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Microsporidia Biological Control Agents and Pathogens of Beneficial Insects

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Chapter 25

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Microsporidian infections of insects are generally chronic, causing subtle pathologies of reduced fecundity and shorter life spans. The lack of acute infections that cause rapid mortality makes microsporidia ill suited as biopesticides for arthropod control. Instead, they are considered to be more useful as long-term regulators of pests and contribute toward the prevention and/or suppression of pest outbreaks.

However, the chronic and debilitating nature associated with the infections makes microsporidia important pathogens of beneficial arthropods that are used for biological pest control. Microsporidia infect beneficial arthropods that regulate pest populations in nature as well as natural enemies that are mass-reared in commercial insectaries. Microsporidia are also common among laboratory-reared beneficial arthropods and often cause chronic disease that reduces host fitness and ultimately affects biological control efficacy.

25.1 MICROSPORIDIA AS BIOLOGICAL CONTROL AGENTS

The utility of microsporidia as biological control agents must be evaluated within the context of the host's ecosystem. Chronic infections and delayed impacts on pest populations may be suitable to natural ecosystems or perennial cropping systems that can accommodate slow population declines or benefit from the mitigation of frequent and/or severe pest outbreaks. The natural regulation of pests typically involves a complex of natural enemies and abiotic factors. Establishment of microsporidian biocontrol agents could be an integral part of the sustained control of a pest population within the appropriate environment and natural enemy complex. In the first section of this chapter, we discuss microsporidian pathogens that have been studied extensively relative to their potential or utilization as biological control agents of rangeland grasshoppers, the European corn borer (ECB), mosquitoes, red imported fire ants, and gypsy moth. Aspects of the microsporidium's pest suppression characteristics are highlighted within the context of the host's ecology. Detailed reviews of the microsporidian pathogens of each of these pests are also listed in Table 25.1.

25.2 RANGELAND GRASSHOPPERS: *PARANOSEMA LOCUSTAE*

The extensive western grasslands of the United States are a region grazed by livestock and plagued by periodic outbreaks of about 10–15 species of grasshoppers (Capinera & Seachrist 1982; Pfadt 2002). The scale of the outbreaks can be uniquely vast, encompassing thousands or even millions of hectares. Because of the numerous species, developmental times, foraging

Microsporidia: Pathogens of Opportunity, First Edition. Edited by Louis M. Weiss and James J. Becnel.

Table 25.1 Characteristics of microsporidia considered or utilized for biological control releases

Species	Targeted Pest	Ecological Host Range	Seasonal Life Cycle Known; Transmission Pathway	Biocontrol Release Type; Outcomes	Detailed Reviews
Paranosema locustae	Rangeland grasshoppers	Wide (122 Orthoptera species)	Yes; horizontal and vertical	Inoculative and inundative; established and spread	Lockwood et al. (1999); Lange and Cigliano (2005)
Nosema pyrausta	European corn borer	Narrow (*O. nubilalis*)	Yes; horizontal and vertical	Natural introduction and inoculative; established and spread	Lewis et al. (2009)
Amblyospora connecticus	Brown salt-marsh mosquito	Narrow (*O. cantator*)	Yes; horizontal and vertical	Experimental augmentation; demonstrated field infection	Andreadis (2007); Solter et al. (2012)
Edhazardia aedis	Yellow-fever mosquito	Narrow (*Ae. aegypti*)	Yes; horizontal and vertical	Experimental augmentation; seminatural field infection and pest suppression	Becnel et al. (2005); Andreadis (2007)
Kneallhazia solenopsae	Red imported fire ant	Narrow (eight *Solenopsis* fire ant species)	No; horizontal and vertical	Natural introduction and inoculative; established and spread	Oi and Valles (2009); Briano et al. (2012)
Vairimorpha invictae	Red imported fire ant	Narrow (three *Solenopsis* fire ant species)	No; horizontal and perhaps vertical	No releases to date	Oi and Valles (2009); Briano et al. (2012)
Nosema lymantriae	Gypsy moth	Narrow (more specific than *V. disparis*)	Yes; horizontal and vertical	Experimental inundative and inoculative; infections detected 3 years	Solter and Hajek (2009); Solter et al. (2012)
Vairimorpha disparis	Gypsy moth	Narrow (less specific than *N. lymantriae*)	No; horizontal	Experimental inundative and inoculative; infections detected but not sustained	Solter and Hajek (2009); Solter et al. (2012)

preferences, aggregation and migration patterns, and other biological and behavioral traits vary greatly. The combination of various biotic traits within a diversity of habitats and weather patterns results in population outbreaks that are unpredictable in intensity and frequency (Lockwood & Lockwood 2008). Destruction of forage can occur rapidly in localized but dispersed foci (Lockwood et al. 2001).

The significant destruction of crops and rangeland forage by grasshoppers (Hewitt & Onsager 1983; Lockwood et al. 2002) have made them the focus of intense control programs utilizing various insecticide formulations (Latchininsky & VanDyke 2006). Generally, insecticides are most efficacious when they target third-instar nymphs to prevent substantial loss of forage and thus must be applied within a limited time frame (Hewitt & Onsager 1983).

Several species of microsporidia have been isolated from various species of grasshopper and locust of which *Paranosema locustae* has been the most extensively evaluated for grasshopper control (Johnson 1997). *P. locustae* which was originally described and named as *Nosema locustae* in 1953 was also assigned to the genus *Antonospora* (Canning 1953; Sokolova et al. 2003; Slamovits et al. 2004), but the genus change was refuted by Sokolova et al. (2005). *P. locustae* infection is best characterized as a chronically debilitating disease associated with reduced feeding, development, and fecundity, in addition to increased mortality rates (Canning 1962; Henry & Oma 1974; Ewen & Mukerji 1980; Johnson & Pavlikova 1986). Sublethal infections in locusts were associated with a shift from the gregarious form to the less damaging solitary phase (Fu et al. 2010). The pathogen mainly infects adipocytes of fat bodies, disrupting metabolism and energy storage. In severe

Figure 25.1 Example of a commercially available formulation of *Paranosema locustae* marketed for the biological control of rangeland grasshoppers. Photo courtesy of G. Merrill/M&R Durango, Inc.

infections, the fat body is greatly hypertrophied with spores, acquiring an opaque, cream coloration which can progress to a pink and eventually a dull-red color (Canning 1953).

P. locustae is transmitted efficiently through ingestion of spores on consumed vegetation, and cannibalism or necrophagy of infected hosts (Canning 1962; Ewen & Mukerji 1980; Henry & Oma 1981). *P. locustae* also can be transmitted transovarially (Raina et al. 1995). However, based on infected hatchlings from field-collected eggs, vertical transmission was considered inadequate to establish infections the following year (Ewen & Mukerji 1980). The third-instar nymph is the host developmental stage most susceptible to infection, with initial mortality peaking during this stage. In addition, a high prevalence of infection is still maintained in survivors (Canning 1962; Henry et al. 1973).

Initially, there was skepticism that *P. locustae* could provide useful microbial control in the field because variability in pathogenicity among host species and slow mortality allowed for continued feeding and crop damage (Canning 1962). However, Henry (1971) reported infection prevalence of 43%, population reductions, and even reduced fecundity when spores were applied in a bait formulation. The chronic debilitative effects, moderate mortality, and efficient transmission made *P. locustae* a plausible augmentative biological control agent (Lockwood et al. 1999). The ability to produce large quantities of spores, albeit in vivo, and transmission requiring relatively few spores (Henry 1971; Henry 1985) were also enticing for the development of *P. locustae* as a microbial insecticide. In 1980, *P. locustae* became the first, and to date only, microsporidium to be a registered and commercially marketed product in the United States (USEPA 2000). It was still being sold in a bait formulation for grasshopper and Mormon cricket control in 2013 (e.g., Nolo Bait™, Semaspore™; Fig. 25.1).

Numerous field studies have examined the efficacy of *P. locustae* in terms of grasshopper population reduction and the more subtle effects on fecundity and seasonal persistence. While some results were encouraging, the presiding trend showed inconsistent intergenerational fecundity reductions, limited interseasonal transmission, and overall low mortality (Lockwood et al. 1999, references therein). Population reductions of 30% accompanied by 20–40% infection prevalence among survivors were typically reported (Johnson 1997). When compared with chemical insecticides that typically achieve 70–95% control, *P. locustae* efficacy would be perceived to be inadequate by end users who desire consistent and economical grasshopper suppression (Vaughn et al. 1991).

Unfortunately, there was an expectation that *P. locustae* could serve as a biocide with fast and extensive efficacy. For a pathogen that causes a slow debilitating disease and eventual death over several weeks, it was a fundamentally unreasonable

goal. However, *P. locustae* could be useful in providing long-term grasshopper suppression in environmentally sensitive areas where rapid and significant pest population reductions at a minimal cost are not the primary concern (Vaughn et al. 1991). Similar sentiments have been reported in China where government-subsidized, large-scale applications were considered inadequate by growers because of low (≤60%) and slow grasshopper mortality (Lockwood et al. 1999). However, *P. locustae* treatments may prove useful if infestations are moderate and sustained infection prevalence contributes to lower populations in subsequent years (Shi et al. 2009).

Refocusing the use of *P. locustae* as a biological component that helps moderate the frequency and severity of grasshopper outbreaks is a more pragmatic use of this organism. Marked reduction (65%) in the recovery of eggs from *P. locustae*-treated plots (Ewen & Mukerji 1980) could impact future grasshopper populations. Bomar et al. (1993) provided evidence of this potential where significant grasshopper population reductions observed in the year of treatment carried over to the second year. In addition, data suggested enhanced egg parasitism possibly due to abnormal egg deposition by *P. locustae*-infected females.

The extensive research and effort in formulation and application technology for *P. locustae* as a biocide also facilitated its implementation as a biological control. Indeed, a bait formulation was used to introduce and establish *P. locustae* into Argentina (Lange & de Wysiecki 1996; Lange & Cigliano 2005; Lange & Azzaro 2008), and bait and spray applications have established it in regions of China (Shi et al. 2009; Miao et al. 2012). It is monitored as a "neoclassical" biological control agent, where an exotic organism is introduced to control a native pest (Lockwood 1993). The establishment of exotics raises concern over impacts on nontarget organisms, shifts in species assemblages, and other components of the ecosystem. *P. locustae* has a very broad host range, infecting 122 species of Orthoptera worldwide (Lange 2005, 2010). After 16 years of monitoring in the Pampas of Argentina, shifts in the relative proportion of grasshopper species were reported but were thought to be part of the inevitable changes to the region as agroecosystems became prevalent (Bardi et al. 2012). Nevertheless, grasshopper outbreaks seemed to have ceased in areas where *P. locustae* established successfully in contrast to similar areas without the microsporidium (Lange & Cigliano 2005, 2010). In China, *P. locustae* applications to expansive study sites (15,000 ha) revealed a minimum 9–10 years of persistence of the pathogen. It was thought that initiating infections of *P. locustae* in large grasshopper populations could result in a sustained presence of the disease which would be important in reducing future outbreaks (Shi et al. 2009; Miao et al. 2012).

25.3 EUROPEAN CORN BORER: *NOSEMA PYRAUSTA*

The ECB, *Ostrinia nubilalis*, was introduced into the United States from Europe in the early 1900s and has spread throughout corn-growing regions in the United States and into Canada. It is a serious pest of corn, where the older-stage larvae burrow into the stalks and tunnel extensively, causing stalk breakage and reduced yields. *Nosema pyrausta* is an obligate intracellular parasite of the ECB and is considered to be an important biotic factor of ECB populations (Lewis et al. 2009). *N. pyrausta* was first described from ECB in 1927 and isolated from ECB in the United States in 1950 (Steinhaus 1952). Subsequent collections of ECB in the United States in 1952 revealed that *N. pyrausta* had spread naturally, being established in numerous counties in seven states (Zimmack et al. 1954). The host range of *N. pyrausta* is apparently very limited as field infections of *N. pyrausta* in Lepidoptera have only been reported from ECB (Lewis et al. 2009).

The effect of *N. pyrausta* on ECB is well documented, causing a chronic, generally nonlethal, debilitation typical of microsporidiosis. Depending on the intensity of the infection and age of the host, *N. pyrausta* caused reductions in egg production and longevity of adults, as well as reduced survivorship among eggs, young larvae, and diapausing larvae (Zimmack & Brindley 1957; Siegel et al. 1986a; Sajap & Lewis 1992). *N. pyrausta* effects on ECB can be minimal with infected larvae developing into viable adults. However, under suboptimal conditions such as cold temperatures, overwintering mortality can be significantly higher in infected larvae (Zimmack & Brindley 1957; Kramer 1959; Solter et al. 1990; Lewis et al. 2009).

N. pyrausta is transmitted both transovarially and horizontally. The efficiency of both modes of transmission is linked to the phenologies of the ECB and its primary host plant, corn. Where ECB has two generations per year, the infection prevalence of the first generation of ECB is dependent on transovarial transmission via infected adults that emerge in the spring after overwintering as diapaused fifth-instar larvae within corn stalk stubble. The infected adults lay infected eggs, which produce the first generation of infected ECB. Horizontal transmission is not as prevalent because of the rapidly growing corn leaves that unfurl and carry defecated spores away from feeding larvae, which remain in the whorl of the corn. In addition, egg laying by ECB that overwintered is relatively synchronous which results in a more uniform age cohort, limiting opportunities for spore ingestion in frass produced by older larvae (Lewis et al. 2009). In contrast, infection prevalence is greater in the second generation as there is greater access to spore-laden frass from the previous and current generations of infected larvae, greater densities of ECB, and interplant movement of ECB larvae (Andreadis 1986; Lewis & Cossentine 1986; Siegel et al. 1988; Lewis et al. 2009). In addition, a parasitoid braconid wasp of ECB, *Macrocentrus cingulum* (=*grandii*), can mechanically transmit *N. pyrausta (*Siegel et al. 1986b), but this mechanism is thought to contribute minimally to infection levels in the field (Andreadis 1986).

Prevalences of *N. pyrausta* among ECB larvae in various studies have fluctuated from 2 to 100% resulting in both enzootic and epizootic phases, and were associated with declines in ECB populations (Hill & Gary 1979; Andreadis 1986; Siegel et al. 1988; Lewis et al. 2006). Despite the sustained presence of *N. pyrausta*, ECB damage still occurs and compels utilization of other control methods. ECB infected with *N. pyrausta* generally exhibit greater mortality when other control methods are implemented. Leaf or sheath collar resistance to ECB feeding in maize in combination with *N. pyrausta* increased ECB mortality (Lewis & Lynch 1976; Lynch & Lewis 1976). Insecticide and spore solutions of *N. pyrausta* applied to growing corn plants resulted in significant reductions in ECB and stalk damage. The effects of the insecticides and *N. pyrausta* were additive and suggested that the two control methods would be compatible components of an ECB pest management program (Lublinkhof et al. 1979; Lublinkhof & Lewis 1980). Unfortunately, biological control agents such as the larval parasitoid, *M. cingulum*, and *Trichogramma* egg parasitoids that parasitize ECB infected with *N. pyrausta* are negatively impacted. Parasitoids that develop in infected ECB have reduced survivorship with *N. pyrausta* infecting parasitoid tissue and affecting host suitability (Andreadis 1982a; Cossentine & Lewis 1987; Sajap & Lewis 1988; Orr et al. 1994; see Sections 25.9.14 and 25.9.16).

The widespread adoption of transgenic corn expressing insecticidal proteins of *Bacillus thuringiensis* (Bt corn) can potentially diminish the prevalence of *N. pyrausta* because its primary host, ECB, is effectively controlled by this technology. Furthermore, *N. pyrausta* spore production was significantly reduced in infected ECB that survived Bt exposure (Pierce et al. 2001). Lewis et al. (2009) contended that *N. pyrausta* will persist as long as refuges of non-Bt corn for resistance management are maintained. Interestingly, studies examining the dynamics of *N. pyrausta* and Bt resistance in ECB revealed that developmental delays due to *N. pyrausta* infection facilitated the synchrony of adult emergence from Bt-resistant and Bt-susceptible ECB populations. This can potentially help maintain Bt-susceptible ECB populations. In addition, *N. pyrausta* infections can further reduce the survivorship of Bt-resistant ECB that feed on Bt corn (Lopez et al. 2010). However, *N. pyrausta* infection was associated with poor dispersal flights by ECB. This may affect the mating/gene flow between Bt-resistant ECB and Bt-susceptible ECB originating from isolated refuges, thereby impacting resistance evolution (Dorhout et al. 2011).

N. pyrausta exhibits ideal attributes of a self-sustaining biological control agent of ECB. Its life cycle and efficient vertical and horizontal transmission integrate well into the phenologies of the ECB and corn. While applications of spore solutions can initiate field infections of *N. pyrausta*, the lack of in vitro mass production methods limits further development as a microbial insecticide (Lewis et al. 2006). Nevertheless, *N. pyrausta* is an important factor regulating ECB populations with epizootics cycling every 6–7 years (Lublinkhof et al. 1979; Lewis et al. 2009). Even with the effectiveness of transgenic Bt corn in suppressing ECB populations, maintaining natural enemies of ECB, like *N. pyrausta*, is important in the event of Bt resistance development and for nontransgenic Bt agroecosytems.

25.4 MOSQUITOES: *AMBLYOSPORA CONNECTICUS* AND *EDHAZARDIA AEDIS*

Microsporidia are pervasive parasites of mosquitoes with over 150 species of microsporidia infecting 14 genera of mosquitoes worldwide (Andreadis 2007). Several of these microsporidia have been thoroughly studied and even released to evaluate their potential as biological control agents. The effects of microsporidian infections on mosquitoes vary widely, but some species are efficiently transmitted and result in epizootics which cause significant larval mortality. Microsporidian infections of mosquitoes also exhibit the typical chronic debilitation and, in some cases, the reduction in fecundity of adult hosts (Andreadis 2007; Solter et al. 2012).

Early attempts at utilizing microsporidia as biological control agents were with *Vavraia* (=*Plistophora*, =*Pleistophora*) *culicis* and *Anncaliia* (=*Nosema*, =*Brachiola*) *algerae* in the South Pacific island of Nauru and in Panama, respectively (Reynolds 1972; Anthony et al. 1978; Vávra & Becnel 2007). Both were spore introductions resulting in infections that reduced adult longevity and fecundity. However, infections did not persist or spread sufficiently to cause significant population declines. Because of their broad host ranges, including vertebrate infections by *A. algerae* (Coyle et al. 2004), these microsporidia are no longer considered candidates as biological control agents (Becnel et al. 2005; Andreadis 2007; Solter et al. 2012). Both species are monomorphic with simple life cycles occurring in a single host and phylogenetically unrelated to a large clade of mosquito-infecting microsporidia. This information raised the possibility that mosquitoes may not be the natural host of these two parasites (Vossbrinck et al. 2004a, 2004b; Becnel et al. 2005).

The large clade of mosquito-infecting microsporidia have complex life cycles that produce several spore types which infect different host tissue, life stages, and generations and for some species require an intermediate host. This complexity suggests coevolution and results in stricter host specificity (Becnel et al. 2005). Elucidation of the complex life cycles not only advanced microsporidiology, but it also rekindled the possibility of the biological control of mosquitoes with microsporidia. These complex life cycles generally have been categorized as either requiring an intermediate host for horizontal transmission, or not requiring an intermediate host and are both horizontally and vertically transmitted.

At least four genera of mosquito microsporidia (*Amblyospora*, *Parathelohania*, *Duboscqia*, *Hyalinocysta*) have obligate, intermediate copepod hosts. In general, for the first three genera listed above, infected mosquito larvae develop into infected adults that transovarially transmit the infection to the next generation of larvae. These larvae die and release meiospores that are ingested by copepods, which also die and release uninucleated spores that are ingested by mosquito larvae that develop into infected adult mosquitoes which continue the cycle. For *Hyalinocysta*, infection does not occur in the adult mosquito. Thus, only horizontal transmission occurs between mosquito larvae and copepods. Infected mosquito larvae, upon their death, release meiospores which are ingested by copepods. The infected copepods release uninucleated spores that are ingested by mosquito larvae (Andreadis 2007).

Extensive studies on *Amblyospora connecticus*, and its host, the brown salt-marsh mosquito, *Ochlerotatus* (=*Aedes*) *cantator*, and intermediate copepod host, *Acanthocyclops vernalis*, revealed consistent seasonal transmission cycles and epizootics. The availability of infectious meiospores from fall epizootics in larval mosquitoes coincides with the appearance of the copepod after summer aestivation. Similarly, horizontal transmission from infected overwintering copepods to mosquito larvae that hatch from overwintering eggs in the spring is critical to sustaining *A. connecticus* (Andreadis 1988, 1990a).

Edhazardia aedis is an example of a mosquito microsporidium with a complex, heterosporous life cycle that does not have an intermediate host. In this species, there is a very efficient transmission both vertically and horizontally in its host, the yellow-fever mosquito, *Aedes aegypti*. Larvae ingest lanceolate, uninucleate spores and develop into infected adults. Binucleate spores are produced after mosquitoes take a blood meal and transovarially transmit *E. aedis* to the next generation. In one study, approximately 95% of the progeny were infected transovarially, and fecundity in infected adults was significantly reduced (Becnel et al. 1995). Infected progeny produce the infective, lanceolate spores that are released upon larval death into the aquatic habitat, where they are ingested by mosquito larvae thereby initiating another cycle. Sometimes the vertical or horizontal cycles can occur repeatedly without alternating, hence providing plasticity to sustain infections (Becnel et al 1989; Becnel & Johnson 2000).

Strong host specificity has been documented for both *A. connecticus* and *E. aedis*. Fifteen species of mosquitoes in five genera were not susceptible to *A. connecticus*. However, four *Aedes* species were infected in the laboratory by oral ingestion of spores. Similarly, *E. aedis* was not infective to 13 mosquito species, but it was horizontally transmitted to 5 other *Aedes* species plus species in 3 other genera. However, transovarial transmission ensued only in their natural hosts *Ae. cantator* and *Ae. aegypti*, for *A. connecticus* and *E. aedis*, respectively. Thus, the microsporidia could only persist over multiple generations in the hosts where their life cycles can be completed both horizontally and vertically (Andreadis 1989a, 1994; Becnel & Johnson 1993).

Both *A. connecticus* and *E. aedis* exhibit desirable biological control traits that include efficient transmission pathways and host specificity. However, few studies have assessed field releases and their resulting persistence and spread. Field introductions of *Amblyospora connecticus* via the release of live, infected copepods have achieved maximum infection rates of 16–24% in its mosquito host, *Ae. cantator* (Andreadis 1989b). Inoculative introductions of *E. aedis*-infected pupae in a seminatural field enclosure resulted in the spread of the microsporidium via transovarial transmission from infected adults, but infections did not reestablish after winter. Inundative introductions of infected larvae in the same setting resulted in the elimination of *Ae. aegypti* populations within 11 weeks (Becnel & Johnson 2000). Beyond these studies, further releases of these promising microsporidians for mosquito biocontrol have not been reported. Nevertheless, the detailed understanding of the seasonal life cycles presents an opportunity to conserve and exploit these microsporidian natural enemies for biological control. For example, timing mosquito larvicide applications to the summer would target uninfected mosquitoes and avoid disrupting the seasonal epizootics of *A. connecticus* (Solter et al. 2012).

25.5 RED IMPORTED FIRE ANTS: *KNEALLHAZIA SOLENOPSAE* AND *VAIRIMORPHA INVICTAE*

The red imported fire ant, *Solenopsis invicta*, is an invasive ant that has become a worldwide concern (Sánchez-Peña et al. 2005; Tschinkel 2006). Its establishment and extensive spread and failed eradication attempts in the United States have brought biological control to the forefront as a viable sustainable strategy that can possibly suppress well-established invasive ants (Williams et al. 2003; Oi & Valles 2009). Among the many natural enemies of *S. invicta*, two microsporidian pathogens from South America, *Kneallhazia solenopsae* and *Vairimorpha invictae*, were considered to be promising biological control agents for this pest ant (Williams et al. 2003). *K. solenopsae* has been utilized in field inoculations in the United States because it was found naturally in *S. invicta* from the United States (Williams et al. 1998), thus easing restrictions on its dissemination.

K. solenopsae, formerly known as *Thelohania solenopsae* (Sokolova & Fuxa 2008), was first observed from *S. invicta* specimens collected in 1973 from Brazil (Allen & Buren 1974). Initial observations reported infected *S. invicta* colonies to be less vigorous, and there were rapid decreases in infected populations when exposed to drought or other stresses (Allen & Buren 1974). More quantitative documentations of *S. invicta* population reductions associated with *K. solenopsae* were later

reported in the United States (Cook 2002; Oi & Williams 2002; Fuxa et al. 2005b) and on the black imported fire ant *Solenopsis richteri* in Argentina (Briano et al. 1995). Population reductions in both hosts fluctuated over time, with maximum reductions of 63% in the United States and 83% in Argentina. Reductions were often attributed to smaller colony sizes as opposed to declines in the number of fire ant nests (Oi & Valles 2009).

Inoculation and infection of laboratory colonies of *S. invicta* indicated that field declines were a result of diminished queen body weight, oviposition rates, and faster death. Significant reductions in brood and overall colony decline were also documented for infected colonies (Williams et al. 1999; Oi & Williams 2002). Both genders of the winged reproductive caste of *S. invicta* also were found infected, and recently mated, infected queens can initiate infected colonies that die sooner than uninfected colonies (Oi & Williams 2003). Evidence of transovarial transmission of *K. solenopsae* has also been reported in *S. richteri* and *S. invicta* (Briano et al. 1996; Valles et al. 2002).

An important factor that mitigates the effect of *K. solenopsae* on field populations of imported fire ants is the number of reproducing queens per ant colony. Colonies that have multiple queens, or polygyne colonies, are not territorial, and colony members can share resources, interchange brood, and move between different nests. In contrast, colonies with only a single queen (monogyne) are territorial with minimal intercolony interactions. *K. solenopsae* is more prevalent in polygyne *S. invicta* populations, although it can be found in monogyne colonies (Oi et al. 2004; Valles & Briano 2004; Fuxa et al. 2005a; Milks et al. 2008). The preponderance of *K. solenopsae* infections in polygyne colonies has been attributed to the nonterritorial behavior which permits movement of infected ants between colonies. There also is a prolonged decline in infected colonies because the interchange of ants allows for an influx of uninfected ants into these colonies. Furthermore, asynchronous infections among the numerous queens within a colony allow for the production of uninfected progeny. The greater intercolony spread and persistent intracolony infections in polygyne populations result in easier detection of *K. solenopsae*. In the territorial monogyne colonies, the infections are isolated, and demise of the single infected queen results in a rapid decline of the colony. This reduces the opportunity for spread and detection of *K. solenopsae* in monogyne populations (Fuxa et al. 2005a; Oi 2006). However, the prevalence of *K. solenopsae* from monogyne *S. invicta* in limited sampling from Argentina was 55%. The reason for the much higher prevalence is unclear, but it was speculated that a genetic basis or perhaps an intermediate host could be responsible (Valles & Briano 2004). Oi and Valles (2009) indicated that the social form assay used by Valles & Briano (2004) may not be completely diagnostic for South American *S. invicta* populations; nevertheless, the assay did provide a conservative determination for monogyny (Shoemaker & Ascunce 2010).

The horizontal transmission pathway of *K. solenopsae* between colonies and even to the queen has not been determined. Four types of spores have been described from *K. solenopsae*-infected ants, but infection through isolated spore inoculation has not been achieved (Shapiro et al. 2003; Chen et al. 2004; Sokolova & Fuxa 2008). Infections of *K. solenopsae* can be established in laboratory and field *S. invicta* colonies by introducing live, infected larvae and/or pupae into the colonies (Williams et al. 1999; Oi et al. 2001, 2008). The introduction of brood was used to establish infections in several states in the United States (Oi & Valles 2009; Fig. 25.2). This method of inoculation suggests brood sharing among polygyne colonies, and brood raiding, or stealing, by monogyne colonies could result in the natural spread of the pathogen (Oi & Williams 2003).

Figure 25.2 Inoculation of a red imported fire ant colony with *Kneallhazia solenopsae*. Live, infected fire ant larvae and pupae (circled) placed inside a nest of fire ants will be adopted into the colony. Photo D. Oi/USDA-ARS.

The host range of *K. solenopsae* has been determined largely from field surveys. Extensive sampling in South America identified *K. solenopsae* in six species of *Solenopsis* fire ants, all in the *Saevissima* species group (Oi & Valles 2009, references therein). In addition, laboratory colony inoculations with live, infected brood and field sampling in *K. solenopsae*-infected areas in Florida did not find infections in 15 non-*S. invicta* ant species (Oi & Valles 2012). However, fire ants in the *geminata* species group, *Solenopsis geminata* and the *S. geminata* × *S. xyloni* hybrid, were infected. Examination of the genetic diversity of the *K. solenopsae*-infecting fire ants in North and South America suggested the existence of variants that may possibly improve biocontrol potential of this pathogen (Ascunce et al. 2010).

Vairimorpha invictae is also considered a possible fire ant biocontrol agent from South America. Its host range is limited to the *Solenopsis saevissima* species group based on field surveys and laboratory inoculations (Briano et al. 2002; Porter et al. 2007; Oi et al. 2010). As with *K. solenopsae*, it is found in all life stages and castes, and human-mediated transmission has not been accomplished with isolated spores (Briano & Williams 2002). However, live brood and live or dead infected adults have been used to initiate infections (Oi et al. 2005). Dead infected workers as inocula, unlike live brood with *K. solenopsae*, can potentially make the logistics of disseminating the pathogen easier.

V. invictae was not as prevalent as *K. solenopsae* in the two imported fire ant species, *S. invicta* and *S. richteri* in South America (Briano et al. 2012). Field populations of *V. invictae*-infected fire ants appear to be sporadic with wide and abrupt fluctuations in prevalence. *K. solenopsae* has a more constant presence with fluctuating yet usually detectible infection levels. Successive epizootics of both pathogens can result in prolonged pressure, or stress, on fire ant populations (Briano et al. 2006). Colony decline was observed to be faster when simultaneous infections of the two microsporidia occurred in the laboratory (Williams et al. 2003). Substantial and sometimes lasting declines in *S. invicta* populations were reported by Briano (2005) in field plots with infections of both *K. solenopsae* and *V. invictae*. *V. invictae* has not been detected in the United States (Oi et al. 2012) and is still being evaluated in quarantine.

25.6 GYPSY MOTH: *NOSEMA LYMANTRIAE* AND *VAIRIMORPHA DISPARIS*

Natural forest ecosystems are complex, consisting of various flora, fauna, climates, topography, and numerous other biotic and abiotic features that interact on micro and regional scales. Within this broad habitat, forest insect outbreaks are a major concern for land managers and have been the subject of intensive study. Among numerous microsporidia reported from forest insects, some microsporidia have been associated with the regulation of these pest population outbreaks (Solter et al. 2012). For example, in the conifer forests of northern North America, *Nosema fumiferanae* is a microsporidium that is considered a regulating component of outbreaks of the eastern spruce budworm, *Choristoneura fumiferana* (Régnière 1984; van Frankenhuyzen et al. 2007). Extensive defoliating outbreaks of this moth occur in 35–40 year cycles (Royama 1984; Boulanger & Arseneault 2004), in which *N. fumiferanae* and other entomopathogens and parasites increase and decrease with host density and can dampen the outbreaks (Eveleigh et al. 2007). In the extended periods between budworm outbreaks, *N. fumiferanae* maintains infection across seasonal generations via transovarial transmission (Eveleigh et al. 2012).

The spruce budworm example given earlier involves an indigenous microsporidium and host in North America. In contrast, the gypsy moth, *Lymantria dispar*, is an invasive pest from Eurasia. This pest is a major defoliator of deciduous hardwood forests in the eastern United States and Canada (Elkinton & Liebhold 1990). In North America, gypsy moth outbreaks cause major regional defoliation every 8–10 years, and there are less extensive outbreaks at 4–5 years of intervals (Johnson et al. 2006). The moth has been the target of a concerted, large-scale campaign to suppress populations and reduce its spread. Insect pathogens are essential components of this program, including microbial insecticides derived from the bacterium *Bacillus thuringiensis kurstaki* (Btk) and the baculovirus *Lymantria dispar* nucleopolyhedrovirus (*Ld*MNPV) which are applied by ground and aerial sprays (McManus & Cóska 2007; Solter & Hajek 2009). A fungal pathogen from Japan, *Entomophaga maimaiga*, has become well established since 1989, causing widespread epizootics that can control gypsy moth populations (Hajek et al. 1990; Elkinton et al. 1991). Microsporidia from gypsy moth populations in Europe are considered to be important natural enemies, and they have been released into the United States as classical biological control agents (Weiser & Novotny 1987; Jeffords et al. 1988; Solter & Becnel 2007).

Introductions of two microsporidian species for gypsy moth in 1986 resulted in the recovery and horizontal transmission of one species, *Nosema portugal*, the following year. It was not detected in a 1989 sampling and apparently has not persisted. Nevertheless, the introductions demonstrated the feasibility of potentially establishing gypsy moth-infecting microsporidia from Europe (Jeffords et al. 1989; Solter & Becnel 2007; Solter et al. 2012). Criteria for regulatory approval to release biological control agents have subsequently become more stringent (Solter & Hajek 2009). The regulatory approval and U.S. release in 2008 of European gypsy moth microsporidia were predicated on a series of detailed studies on taxonomy, host specificity, tissue tropism, and transmission that can serve as a model for evaluating microsporidian biological control candidates.

In Europe, several species of native microsporidia are prevalent in gypsy moth populations where they maintain a low presence in the intervals between outbreaks, then increase as the moths progress toward outbreak densities

(McManus & Solter 2003). The early taxonomy of these microsporidia was unclear partially due to unknown spore dimorphism. More recent analyses have clarified taxonomic placements with four species, *Nosema lymantriae* (includes two isolates), *Nosema portugal*, *Endoreticulatus schubergi*, and *Vairimorpha disparis*, being the subject of transmission and host specificity studies to assess their biological control potential (Solter et al. 1997; Vávra et al. 2006; Solter & Hajek 2009; Solter et al. 2012). Determining the identity of the various microsporidia reported to infect gypsy moth was critical for providing a link to early research with contemporary studies and to accurately identify relevant species.

Determining host specificity of potential biological control agents is crucial for selecting agents for release. Solter et al. (1997) described how categorizing responses in nontarget Lepidoptera challenged with gypsy moth microsporidia as either (i) refractory, (ii) atypical with few environmental spores, or (iii) heavy infections subdivided into (a) heavy atypical infections or (b) patent, "host-like" infections, provided a more informative assessment of the potential ecological host range. For instance, physiological host specificity based solely on the presence or absence of infection among nontarget hosts without accounting for indicators of a patent infection, such as spore production and viability, would exclude a majority of potential biocontrol agents (Solter & Maddox 1998a).

The narrower ecological, or field, host range is also supported by field host surveys in Europe. Surveys of gypsy moth and other Lepidoptera in Bulgaria found that the gypsy moth microsporidia, *N. lymantriae*, *V. disparis*, and *E. schubergi*, were only observed in gypsy moth and not in any of the other Lepidoptera. Similar specificity was reported in Slovakia where spore suspensions of *N. lymantriae* and *V. disparis* were sprayed onto foliage and resulted in limited nontarget infections that did not persist (Solter et al. 2000, 2010).

In addition to evaluating host specificity, understanding the transmission pathways of the various gypsy moth microsporidia facilitated the assessment of other criteria, such as virulence and infectivity useful for the selection of biological control agents. Transmission of *E. schubergi* is through the ingestion of defecated spores that contaminate foliage. This reflects the midgut site of infection of *E. schubergi*. Infectivity of *E. schubergi* spores collected from the feces 10–25 days after inoculation was 100%, yet virulence was low with a maximum of 38% larval mortality under laboratory conditions (Goertz & Hoch 2008a). Survivorship of infected larvae to adulthood, transovum (but not transovarial) transmission, venereal transmission, and the infectivity of spores from overwintered cadavers contribute to the cross-generational persistence of *E. schubergi* (Goertz & Hoch 2008b). Laboratory host specificity studies indicated that *E. schubergi* was a generalist, with 15 of 33 nontarget species exhibiting patent infections. As such, *E. schubergi* was not a good candidate for release as a classical biological control agent (Solter et al. 1997, 2012).

Nosema lymantriae infects Malpighian tubules, fat body, silk glands, and gonads. The broad tissue tropism results in horizontal transmission via feces laden with spores probably from the Malpighian tubule infection (Goertz & Hoch 2008a). The systemic infection that extends into the other tissue such as fat body also contributes to significant dissemination of spores from decaying cadavers. Transovarial transmission also occurs (Goertz & Hoch 2008b) as a likely result of gonad infection. The moderate virulence apparently permits the combination of horizontal and vertical transmission, which allows for effective transmission as well as persistence to the next generation of hosts (Goertz & Hoch 2008b).

Vairimorpha disparis infections initiate in the midgut then progresses primarily to fat body. It is a virulent pathogen that usually causes larval death regardless of spore dosage and larval age. Dissemination of infective environmental spores is primarily through cadaver decomposition. Spores can be excreted with the feces prior to larval death, but it is a minor transmission pathway (Goertz & Hoch 2008a). There is no evidence for transovum infection with *V. disparis* (Goertz & Hoch 2008b). The high virulence, lack of vertical transmission, and poor overwintering viability of spores have not indicated a definitive mechanism for interseasonal or generational persistence of *V. disparis*. Nevertheless, *V. disparis* has been recovered over successive years at a single site in Bulgaria (Solter et al. 2000).

Inoculative releases of *N. lymantriae* and *V. disparis* for classical biological control have been made in Bulgaria and the United States by introducing infected third-instar gypsy moth larvae. In Bulgaria, releases of *N. lymantriae* in 2008 resulted in 55% recovery of infected larvae that year, followed by detections of 7–10% in the three subsequent years. In the United States, *N. lymantriae* was not detected in gypsy moth larvae in the year following releases in 2008 and 2010. Yet, 12.5% infection was found in 2010. For the *V. disparis* releases in Bulgaria in 2008 and 2010, infections of 57 and 4.3% were reported in the release year, respectively. However, *V. disparis* was not detected a year after each release. In the United States, *V. disparis* was not detected in each year following the releases in 2008 and 2010. However, 27% *V. disparis* infection was found in 2010. Confounding the recovery of both microsporidia was low gypsy moth population densities. In the United States, *E. maimaiga* may have contributed to the decline in gypsy moths. Neither *N. lymantriae* nor *V. disparis* were detected in any nontarget larvae sampled during the monitoring periods at any of the sites in Bulgaria and the United States, thus further validating their host specificity (Solter, personal communication). Certainly, the effect of other entomopathogens like *E. maimaiga* on microsporidian establishment must be further evaluated. Yet the greater field recovery of *N. lymantriae* suggests the importance of multiple transmission pathways within a species in establishing microsporidia as biological control agents.

25.7 CONCLUDING REMARKS

A recurrent theme among the microsporidia considered or utilized for biological control is the need to understand their seasonal life cycles and transmission pathways within their natural environment. This knowledge provides a basis for selecting biocontrol agents and devising release and conservation methods to facilitate their establishment (Andreadis 1990b). These microsporidia also shared several traits amenable for biological control including (i) efficient vertical and horizontal transmission which results in consistent infection and dispersal, (ii) moderate virulence whereby there is a balance between host mortality and pathogen persistence/recycling, (iii) a host range restricted to the specific pest or closely related pest species, and (iv) practical inoculation or release methods (Table 25.1). Given that microsporidia have yet to be mass-produced in vitro, inoculative or augmentative releases that rely on self-sustaining persistence and natural spread are the most plausible approaches to utilizing these natural enemies for biological control.

With the continued globalization of commerce, the increase in worldwide travel, and the alteration of natural habitats, introductions of new invasive species and the outbreaks of formerly benign pests will certainly occur. Establishment and/or manipulation of natural enemies to regulate populations of these new pests are probably the most sustainable pest suppression approaches to achieve tolerable pest levels. Establishment and natural spread of a single biological control agent that completely controls a pest is a coveted goal. However, natural population regulation is more likely a complex interaction of several natural enemies, other biotic factors, and abiotic conditions. Among the microsporidia that have been utilized or evaluated for biological control, they all operate within character as causing chronic, debilitating infections often with host density-dependent prevalence. Hence, microsporidia-associated pest population declines are usually not sufficient by themselves to satisfactorily reduce a pest population. Yet, in unmanaged or natural habitats, they are well suited to contribute toward alleviating pest outbreaks and suppressing reservoirs of migrating pests (Bomar et al. 1993; Becnel & Johnson 2000; McManus & Solter 2003; Oi et al. 2008; Lewis et al. 2009). Thus, reasonable expectations for microsporidian biological control should be recognized with the realization that they can be an important component of pest population regulation.

25.8 MICROSPORIDIA AS PATHOGENS OF BENEFICIAL INSECTS

As common pathogens of insects, microsporidia infect several species of beneficial arthropods including those that are field-collected or mass-reared in insectaries for biological pest control as well as those that provide pest control in nature. Microsporidia are also common among laboratory-reared beneficial arthropods and often cause chronic disease that reduces host fitness and ultimately affects biological control efficacy.

Due to the cryptic nature of microsporidia, infected beneficial arthropods may only be discovered during routine examination of specimens for pathogens. In many cases, microsporidia-infected beneficial arthropods exhibit a variety of symptoms, including reduced food consumption, prolonged larval and pupal development, deformed pupae and adults, reduced fecundity and longevity, and death (e.g., see Brooks & Cranford 1972; Siegel et al. 1986b; Zchori-Fein et al. 1992; Geden et al. 1995; Bjørnson & Keddie 1999; Schuld et al. 1999; Idris et al. 2001; Steele & Bjørnson 2012). Microsporidia may have more noticeable effects when host insects are under stress (Kluge & Caldwell 1992), as is often the case in mass-rearings.

The following is a summary of microsporidia in field-collected and mass-reared beneficial insects used for biological pest control as well as infections in beneficial insects that control pest insect populations in nature. A summary of the microsporidian pathogens reported from beneficial arthropods is listed in Table 25.2.

25.9 PARASITOIDS

25.9.1 *Apanteles fumiferanae* (Hymenoptera: Braconidae)

Apanteles fumiferanae is a solitary endoparasitoid of the spruce budworm, *Choristoneura fumiferana*, in Canada, and in any given year, between 10 and 30% of natural spruce budworm populations are parasitized (see Nealis & Smith 1987). The microsporidium *Nosema* (formerly *Perezia*) *fumiferanae* infects much of the spruce budworm population in southern Ontario, but the frequency of infection seems to increase with high densities of spruce budworm in localized areas (see Nealis & Smith 1987). *N. fumiferanae* infects several tissues of spruce budworm larvae and adults (Thomson 1955; Percy 1973), but the effects of this pathogen on its host depend upon the intensity of infection. Low to moderate infections prolong larval growth and development and reduce adult fecundity (Thomson 1958b), whereas heavy infections kill the host (Wilson 1985). Occasionally, both the parasitoid and the microsporidian pathogen are observed in a single host (Thomson 1958a).

Spores of *N. fumiferanae* are confined to the gut contents of *A. fumiferanae* larvae but do not invade any tissues (Thomson 1958a; Nealis & Smith 1987). *A. fumiferanae* larvae have a blind gut where food is stored to last through pupation. When

Table 25.2 Beneficial arthropods and nematodes infected with microsporidia

Parasitoids (All Hymenoptera Except as Noted)	Parasitoid Host	Microsporidium	Microsporidium Impact on Beneficial
Apanteles fumiferanae (Braconidae)	Spruce budworm, *Choristoneura fumiferana*	*Nosema fumiferanae*	Limited adverse effect
Ascogaster quadridentata (Braconidae)	Coddling moth, *Laspeyresia pomonella*	*Nosema carpocapsae*	Deleterious
Asobara tabida (Braconidae)	*Drosophila*	*Tubulinosema kingi*	Deleterious
Pachycrepoideus vindemiae (Ichneumonidae)			Deleterious
Bonnetia comta (Diptera: Tachinidae)	Black cutworm, *Agrotis ipsilon*	*Vairimorpha necatrix*; *Vairimorpha* sp.	Deleterious Limited adverse effect
Bracon mellitor (Braconidae)	Boll weevil, *Anthonomus grandis*	*Glugea gasti*	Deleterious
Campoletis sonorensis (Ichneumonidae)	Corn earworm, *Helicoverpa zea*	*Nosema heliothidis*	Limited adverse effect
Cardiochiles nigriceps (Braconidae)	Tobacco budworm *Heliothis virescens*	*Nosema campoletidis*; *Nosema cardiochiles*	
Catolaccus aenoviridis *Spilochalcis side* (hyperparasitoids)	*C. sonorensis*	*Nosema heliothidis* *Nosema campoletidis*	Minor/no effect
Cotesia flavipes (Braconidae)	Spotted stem borer, *Chilo partellus*	*Nosema partelli* *Nosema bordati*	Deleterious
Cotesia flavipes	Sugarcane borer, *Diatraea saccharalis*	*Nosema* sp.	Deleterious
Cotesia glomerata (Braconidae)	Imported cabbage worm, *Pieris rapae* Cabbage white, *Pieris brassicae*	*Nosema mesnili*	Deleterious
Cotesia marginiventris (Braconidae)	Lawn armyworm, *Spodoptera mauritia acronyctoides*	*Nosema* sp.	Deleterious
	Corn earworm, *H. zea*	*Vairimorpha* sp.	None
Dahlbominus fuscipennis (Eulophidae)	Larch sawfly, *Pristiphora erichsonii*	*Thelohania pristiphorae*	Unknown
Diadegma semiclausum (Ichneumonidae)	Diamondback moth, *Plutella xylostella*	*Vairimorpha imperfecta*	Deleterious
Encarsia nr. *pergandiella* (Aphelinidae)	Silverleaf whitefly, *Bemesia argentifolii*	*Nosema* sp.	Deleterious
Glyptapanteles liparidis (Braconidae)	Gypsy moth, *Lymantria dispar*	*Vairimorpha disparis*	Deleterious
Lydella thompsoni (Diptera: Tachinidae)	European corn borer, *Ostrinia nubilalis*	*Nosema pyrausta* *Nosema* sp.	None Deleterious
Macrocentrus ancylivorus (Braconidae)	Oriental fruit moth, *Grapholitha molesta*	*Nosema destructor*	Deleterious
Macrocentrus cingulum (Braconidae)	European corn borer, *Ostrinia nubilalis*	*Nosema pyrausta* *Nosema* sp. *Vairimorpha necatrix*	None Deleterious Deleterious
Muscidifurax raptor (Pteromalidae)	Housefly, *Musca domestica* Stable fly, *Stomoxys calcitrans*	*Nosema muscidifuracis*	Deleterious
Pediobius foveolatus (Eulophidae)	Mexican bean beetle, *Epilachna varivestis*	*Nosema epilachnae* *Nosema varivestis*	Deleterious
Tachinaephagus zealandicus (Encyrtidae)	Muscoid fly larvae	*Nosema* sp.	Deleterious
Trichogramma chilonis (Trichogrammatidae)	Cabbage moth, *Plutella xylostella*	*Vairimorpha* sp.	Deleterious

(*Continued*)

Table 25.2 (*Continued*)

Parasitoids (All Hymenoptera Except as Noted)	Parasitoid Host	Microsporidium	Microsporidium Impact on Beneficial
Trichogramma evanescens *Trichogramma nubilale* (Trichogrammatidae)	European corn borer, *Ostrinia nubilalis*	*Nosema pyrausta*	Deleterious
Phytophagous insects (all Coleoptera except as noted)			
Galeruca rufa (Chrysomelidae)	Field bindweed, *Convolvulus arvensis*	*Nosema* sp.	Deleterious
Lema cyanella (Chrysomelidae)	Canada thistle, *Cirsium arvense*	*Nosema* sp.	Deleterious
Rhinocyllus conicus (Curculionidae)	Italian thistle, *Carduus tenuiflorus*	*Nosema* sp.	Unknown
Crown weevil, *Ceutorhynchus litura* (Curculionidae); Canada thistle stem gallfly, *Urophora cardui* (Diptera: Tephritidae)	Canada thistle, *Cirsium arvense*	*Nosema* sp.	Unknown
Predators			
Adalia bipunctata, two-spotted lady beetles (Coleoptera: Coccinellidae)	Aphids	*Tubulinosema hippodamiae* *Undescribed microsporidium*	None Deleterious
Chrysoperla californica; *Chrysoperla carnea* (Neuroptera: Chrysopidae)	Aphids	*Pleistophora californica* *Nosema pyrausta*	Deleterious None
Coccinella septempunctata, seven-spotted lady beetle and other lady beetles (Coleoptera: Coccinellidae)	Aphids	*Nosema tracheophila* *Nosema coccinellae*	Tissues infected, effects not reported
Hippodamia convergens, convergent lady beetle (Coleoptera: Coccinellidae)	Aphids	*Nosema hippodamiae* *Tubulinosema hippodamiae*	Not reported Deleterious
Metaseiulus occidentalis (Acari: Phytoseiidae)	Spider mites	*Oligosporidium occidentalis*	Deleterious
Neoseiulus cucumeris; *Neoseiulus barkeri* (Acari: Phytoseiidae)	Western flower thrips, *Frankliniella occidentalis*; onion thrips, *Thrips tabaci*	Possibly a Pleistophoridae *Nosema steinhausi* Another unidentified	Not reported
Forage mites *Acarus siro*; grain mite *Tyrophagus putrescentiae*, used as food in beneficial mite mass-rearing		*Nosema steinhausi* *Intexta acarivora*	Not reported
Phytoseiulus persimilis (Acari: Phytoseiidae)	Spider mites	*Microsporidium phytoseiuli* Two undescribed microsporidia	Deleterious Not reported
Sasajiscymnus tsugae (Coleoptera: Coccinellidae); *Laricobius nigrinus* (Coleoptera: Derodontidae)	Hemlock woody adelgid	*Tubulinosema* sp. Several *Nosema* spp.	Deleterious
Other beneficial invertebrates			
Steinernema glaseri (Rhabditida: Steinernematidae)	Beetle grubs	Undescribed microsporidium	Deleterious
Steinernema carpocapsae (Rhabditida: Steinernematidae)	Lepidopteran larvae	*Pleistophora schubergi*; *Nosema mesnili*	Unknown
Xysticus cambridgei (Araneae: Thomisidae)	Spider prey	*Oligosporidium arachnicolum*	Unknown

A. fumiferanae larvae develop in *N. fumiferanae*-infected hosts, the large numbers of ingested spores acquired during feeding accumulate in the gut until it is filled almost entirely with spores. These spores are both indigestible and unable to infect the parasitoid. However, the accumulation of spores reduces the ability of the parasitoid larvae to store food, and they become susceptible to starvation. Although microsporidian spores are observed in association with *A. fumiferanae* adults,

these are not found on parasitoid adults that have been washed in detergent and saline. It is likely that spores are only on the external surface of adult parasitoids (Nealis & Smith 1987).

Microsporidian spores appear to be carried on the outside of female *A. fumiferanae* adults but not males. Despite this observation, pathogen prevalence and intensity of *N. fumiferanae* are low, and it is unlikely that the parasitoid is an important vector of the pathogen in spruce budworm populations. There is also little evidence that the microsporidium and the parasitoid are associated in natural populations of spruce budworm at a level that would be necessary for successful horizontal or vertical pathogen transmission (Nealis & Smith 1987).

Despite not invading the larval parasitoid tissues, the microsporidian pathogen does adversely affect larval pupation. Higher proportions of parasitoid larvae that emerge from infected budworm hosts do not complete development when compared to those that emerge from uninfected hosts. The microsporidium causes significant larval mortality when heavily infected spruce budworm hosts die as a result of infection (Thomson 1958a; Nealis & Smith 1987). The duration of within-cocoon development, cocoon weights, and adult longevity of *N. fumiferanae*-infected parasitoids do not differ from those of uninfected hosts (Nealis & Smith 1987). Parasitoid mortality is likely to occur when the host has extremely high infection levels, but the effects of the pathogen are lessened because *A. fumiferanae* emerges from spruce budworm at an early stage of host development. Within-host development of *A. fumiferanae* in heavily infected hosts seems to be unimportant, and mass-rearings of the parasitoid are unlikely to be affected by moderate levels of microsporidia in host colonies (Nealis & Smith 1987).

In the laboratory, both pathogen prevalence and intensity of *N. fumiferanae* infections increase over time, but these increases are less in hosts that are parasitized by *A. fumiferanae* when compared to those that are not. The inability of the pathogen to infect larval and adult *A. fumiferanae*, and the lack of evidence that there is a significant interaction between the pathogen and parasitoid in natural spruce budworm populations, has led to the conclusion that each act as independent mortality factors (Nealis & Smith 1987).

25.9.2 *Ascogaster quadridentata* (Hymenoptera: Braconidae)

The braconid *Ascogaster quadridentata* is an endoparasitoid of the coddling moth, *Laspeyresia pomonella.* The microsporidium *Nosema carpocapsae* infects coddling moth larvae, pupae, and adults as well as *A. quadridentata* larvae. The pathogen infects only the anal vesicle of first-instar larvae, but the final larval instar acquires *N. carpocapsae* spores as it feeds on the fat body of its host. These spores accumulate within the larval blind midgut, and parasitoid tissues are infected as the larva emerges from the host to spin its cocoon. The pathogen infects the pupal midgut epithelium, silk glands, and Malpighian tubules, and heavily infected pupae often do not develop into adults. Adults that eclose rarely mate and their longevity is reduced (Huger & Neuffer 1978).

25.9.3 *Asobara tabida* (Hymenoptera: Braconidae)

The microsporidium *Tubulinosema kingi* infects laboratory colonies of *Drosophila*. The pathogen prolongs prepupal and pupal development, increases pupal mortality, and reduces adult longevity and fecundity. Horizontal transmission of *T. kingi* is 100% when *Drosophila* larvae feed on media that includes spores from infected, dead flies. However, both horizontal transmission of the pathogen among larval cohorts and vertical transmission are low (<10%) (Futerman et al. 2006).

In 2001, Futerman et al. (2006) noticed pale and distended abdomens in relatively high numbers of the larval and pupal parasitoids, *Asobara tabida* and *Pachycrepoideus vindemiae* (Ichneumonidae). Examination of symptomatic parasitoids revealed *T. kingi* spores (Futerman et al. 2006). These were diplokaryotic, oval, or slightly pyriform and measure $4.6 \pm 0.3 \times 2.7 \pm 0.1\,\mu m$ (mean ± SD, fresh smears) and $3.6 \pm 0.3 \times 2.4 \pm 0.2\,\mu m$ (fixed smears) (Franzen et al. 2006; Futerman et al. 2006).

Futerman et al. (2006) examined the effects of *T. kingi* on *Asobara tabida* by allowing microsporidia-free parasitoids to oviposit in *D. subobscura* that were reared on media to which *T. kingi* spores (~2.5×10^6 and 2.5×10^4 in 0.1% SDS) were added. The pathogen caused an increase in pupal mortality and reduced egg load, both of which increased with an increase in spore dose. Progeny production of infected *A. tabida* is also reduced when compared to uninfected controls.

Although pathogen transmission from infected *D. subobscura* to *A. tabida* is 100%, infected *A. tabida* adults do not transmit the pathogen vertically, nor do they transmit the pathogen to additional *D. subobscura* larvae. *T. kingi* also infects *Pachycrepoideus vindemiae*, resulting in high mortality (Futerman et al. 2006).

The effects of *T. kingi* are more pronounced with respect to the fitness of *A. tabida* and *P. vindemiae* as opposed to that of the host. The authors offer several possible explanations for this observation. In general, parasitoids may be in contact with high pathogen densities as they develop within infected host tissues and the duration of parasitoid development exceeds that of the host, allowing the pathogen more time to develop in parasitoid tissues. The effects of *T. kingi* are more pronounced with respect to the parasitoid than for *D. subobscura* (Futerman et al. 2006).

25.9.4 *Bonnetia comta* (Diptera: Tachinidae)

Early-stage black cutworm, *Agrotis ipsilon* larvae become infected when exposed to *Vairimorpha necatrix* and *Vairimorpha* sp. spores. These pathogens damage the cutworm gut and may result in the rapid death of black cutworm larvae.

Cossentine and Lewis (1986) studied the impact of *V. necatrix* and *Vairimorpha* sp. on *Bonnetia comta*, a tachinid parasitoid of *A. ipsilon* larvae. *B. comta* was allowed to parasitize and develop within *A. ipsilon* larvae that were fed a diet to which either *V. necatrix* or *Vairimorpha* sp. spores were added. When *B. comta* develop within *V. necatrix*-infected *A. ipsilon* larvae instead of healthy hosts, the number of parasitoids that complete pupation is reduced, as are the puparia weights and the duration for adult parasitoid eclosion. The same trends are observed when hosts are infected with *Vairimorpha* sp., but these observations are not significant.

When infected with *V. necatrix* and *Vairimorpha* sp., the indigestible microsporidian spores accumulate within the gut lumen of the parasitoid, which becomes greatly distended as the *B. comta* larvae mature. The accumulation of spores and associated tissue damage are thought to result in nutrient deficiency. Both pathogens have a greater detrimental effect on female parasitoids in comparison to males, and the effect of these pathogens increases when host larvae are infected with higher concentrations of spores (Cossentine & Lewis 1986).

25.9.5 *Bracon mellitor* (Hymenoptera: Braconidae)

The ectoparasitoid *Bracon mellitor* parasitizes a wide range of coleopteran and lepidopteran hosts and is the primary parasitoid of the cotton boll weevil, *Anthonomus grandis* (see Tillman & Cate 1989). McLaughlin (1969) described the microsporidium, *Glugea gasti*, from the boll weevil. The pathogen initially infects the alimentary canal but later proliferates throughout most tissues. Mature spores measure $4.3 \pm 0.3 \times 2.3 \pm 0.2\,\mu m$. A second, undescribed microsporidium was later detected in weevil rearings. Because the boll weevil is used as a host to rear the braconid, *Bracon mellitor*, a study was undertaken by Bell and McGovern (1975) to determine the effects of *G. gasti* and the undescribed microsporidium on *B. mellitor*.

B. mellitor did not emerge from weevil larvae infected with *G. gasti*; however, adult emergence from hosts infected with the undescribed microsporidium did not differ from the control. Adult *B. mellitor* that emerge from hosts infected with the undescribed pathogen do not contain microsporidian spores. However, examinations of dead *B. mellitor* immatures recovered from hosts infected with *G. gasti* contain immature stages of the pathogen and many spores. The authors conclude that infection of *A. grandis* by *G. gasti* would have an adverse effect on *B. mellitor* mass-rearings, whereas the undescribed microsporidium would have little or no effect (Bell & McGovern 1975).

25.9.6 *Campoletis sonorensis* (Hymenoptera: Ichneumonidae) and *Cardiochiles nigriceps* (Hymenoptera: Braconidae)

The microsporidium *Nosema heliothidis* is a pathogen of the corn earworm, *Helicoverpa* (=*Heliothis*) *zea* and tobacco budworm *Heliothis virescens*. Brooks and Cranford (1972) report the effects of *N. heliothidis* on the larval parasitoids *Campoletis sonorensis* and *Cardiochiles nigriceps* and describe the microsporidian pathogens, *Nosema campoletidis* and *Nosema cardiochiles* from *C. sonorensis* and *C. nigriceps*, respectively. McNeil and Brooks (1974) report the effects of *N. heliothidis* and *N. campoletidis* on *Catolaccus aenoviridis* and *Spilochalcis side*, two hyperparasitoids of *C. sonorensis*.

When *C. sonorensis* develops within a *N. heliothidis*-infected *H. zea* larva, all stages of the parasitoid develop systemic infections. *C. sonorensis* larvae ingest microsporidia-infected host tissues during development, and microsporidian spores accumulate within the larval blind gut. In most cases, these spores are confined to the lumen of the larval midgut or in regions of the midgut epithelial cells, but in some larvae, the pathogen invades tissues that are adjacent to infected areas of the midgut, including the Malpighian tubules, silk glands, muscles, and fat body. The gonads and nervous system remain uninfected. Masses of spores in the larval meconium are excreted during pupation. The tissues of pupae tend to be more heavily infected than those of the larvae. Microsporidian spores are observed in the midgut, fat body, muscles, and nerves. The epidermal cells, tracheal cells, and the epithelial sheaths of the ovaries and testes are also infected. Adults are more heavily infected than the pupae. Adult reproductive tissues are more extensively invaded, providing evidence that *N. heliothidis* is transmitted transovarially (Brooks & Cranford 1972).

The majority of parasitoids that develop within *N. heliothidis*-infected host larvae eclose successfully and most appear normal. A few adults have malformed wings and some individuals are weak upon emergence. The latter usually die within 24 h (Brooks & Cranford 1972).

Neither of the hyperparasitoids *C. aenoviridis* or *S. side* are infected after developing in *N. heliothidis*-infected *C. sonorensis* pupae. *N. heliothidis* spores accumulate in the blind midguts of hyperparasitoid larvae, but these are egested with the meconium during pupation. Neither adult nor pupae of either hyperparasitoid become infected with the microsporidium, and as a result, the pathogen has no effect on the development or longevity of *C. aenoviridis* or *S. side* (McNeil & Brooks 1974).

The microsporidium *Nosema campoletidis* infects *C. sonorensis*, and pathogen prevalence is relatively high (7.9–38.5%) in natural populations of the parasitoid in North Carolina. It is thought that this is due to the high rate of transovarial transmission of the pathogen. Spores of *N. campoletidis* are ovocylindrical and measure 3.1–6.2 × 1.4–2.4 µm (fresh; mean = 4.75 × 1.78 µm). In mature *C. sonorensis* larvae, infection is systemic, and spores invade the nerves, larval gonads, midgut epithelial cells, Malpighian tubules, muscles, tracheal and epidermal cells, silk glands, and fat body. However, the gut lumen is almost free of spores. In adults, infection is generally heavy, and spores are observed in the midgut epithelial cells and midgut lumen, ganglia, ovaries and testes. Despite the systemic infection caused by *N. campoletidis*, the pathogen had no measurable effect on the duration of development and no effects on adult mating, fecundity, or longevity. There are no signs associated with infection, and field-collected adults cannot be distinguished from healthy parasitoids (Brooks & Cranford 1972).

Adults of the hyperparasitoid *C. aenoviridis* are infected systemically when they develop within *N. campoletidis*-infected *C. sonorensis* pupae. The pathogen infects the intestinal epithelia, Malpighian tubules, and fat body of both pupae and adults, and transovarial transmission is observed. The hyperparasitoid *S. side* is also infected by *N. campoletidis*, but in this case, development of the microsporidium ceases during the sporoblast stage. Infection is limited to the midgut epithelial cells, and mature spores do not develop. In the case of both *C. aenoviridis* and *S. side*, there are no signs associated with infection, and detrimental effects caused by the pathogen are presumed to be minor (McNeil & Brooks 1974).

The microsporidium *Nosema cardiochilis* infects *C. nigriceps*, a braconid endoparasitoid of *H. virescens*. Pathogen prevalence in *C. nigriceps* larvae and adults from tobacco fields is relatively common (up to 33.3%). The presence of *N. cardiochiles*-infected early-stage larvae in healthy field-collected *H. virescens* larvae suggests that the pathogen is transmitted transovarially. Infected, field-collected specimens have no signs associated with infection (Brooks & Cranford 1972).

N. cardiochilis spores are often ovocylindrical and slightly curved. They measure 3.6–6.0 × 1.4–2.4 µm (fresh; mean = 4.78 × 1.75 µm). Infection of *C. nigriceps* larvae is generally heavy, and systemic. *N. cardiochiles* spores are observed in the epithelial cells of the midgut and hindgut, epidermal and tracheal cells, fat body, oenocytes, silk glands, brain and ventral ganglia, muscles, and developing gonads. In infected adult females, the Malpighian tubules are infected, and the oenocytes, adipose tissue, and reproductive organs are heavily infected. Parasitoid eggs contain *N. cardiochilis* spores (Brooks & Cranford 1972).

The pathogen has no effect on the duration of development of the parasitoid, but a significant portion of adults (23.1%) did not eclose after they emerged as prepupae from infected host larvae. Under laboratory conditions, the pathogen may limit production of parasitoids if they develop within *Nosema*-infected hosts (Brooks & Cranford 1972).

25.9.7 *Cotesia* (*Apanteles*) *flavipes* (Hymenoptera: Braconidae)

Cotesia (formerly *Apanteles*) *flavipes* is a gregarious, larval parasitoid of lepidopteran stem borers. *C. flavipes* has a wide host range and has been released for biological control of stem borers throughout the world (see Bordat et al. 1994). This parasitoid has been introduced to the Comoros Archipelago and into South Africa for control of the spotted stem borer, *Chilo partellus* (see Bordat et al. 1994; Kfir & Walters 1996), where *Nosema bordati* is known to infect this same host.

In 1989, high mortality was observed in laboratory colonies of *C. flavipes*. Examination of dead parasitoid larvae and pupae revealed numerous *Nosema partelli* spores. This pathogen also infects *Chilo partellus*. Although several parasitoids become infected after being reared on *N. partelli*-infected *C. partellus* larvae, mortality is observed only in the braconid parasitoids *C. flavipes* and *Allorhogas pyralophagus*.

Bordat et al. (1994) investigated the potential relationships between *C. flavipes* and *N. bordati* when they are simultaneously present in *Chilo partellus*. Spore dilutions (ranging from 7.59×10^3 to 7.59×10^7 spores/ml) obtained from ground and homogenized *C. partellus* tissues were administered *per os* to *C. partellus* larvae. Percent mortality of first-instar *C. flavipes* larvae increased with an increase in spore dose. *N. bordati* caused 78.8–97.7% mortality in immature instars of *C. flavipes*, and adult parasitoids that eclose successfully from immatures that are fed these spore doses die prematurely. The fecundity and capacity for infected adults to parasitize the host are reduced.

Infection in *C. flavipes* larvae begins in the anal vesicle and progresses to the gut epithelium, nervous system, and musculature, which become heavily infected. The reproductive organs of both male and female *C. flavipes* are infected with spores, indicating that females with light infections of *N. bordati* transmit the pathogen vertically. Based on the prevalence and effects of *Nosema bordati* in *C. flavipes*, the pathogen is responsible, in part, for the ineffective control of *Chilo partellus* in the Comoros (see Bordat et al. 1994).

C. flavipes is also an important natural enemy of the sugarcane borer, *Diatraea saccharalis*. Parasitoids are mass-reared in *D. saccharalis* larvae under laboratory conditions for augmentative release against this pest on sugarcane. High prevalence of *Nosema* sp. in laboratories that produce *C. flavipes* led Simões et al. (2012) to investigate the impact of this pathogen on *C. flavipes*. The parasitoid becomes infected with *Nosema* sp. when developing inside infected *D. saccharalis* host larvae. Heavy infections cause host mortality, and in many cases, this prevents *C. flavipes* from completing its life cycle. The pathogen prolongs larval and pupal development, and decreases both adult longevity and the number of offspring produced.

C. flavipes females are more attracted to plants that have been fed upon by *D. saccharalis* than those that have not; however, *Nosema* sp.-infected females are less able to make this distinction. Once infected, the parasitoid transmits the pathogen to uninfected host larvae. The authors conclude that the effects of *Nosema* sp. in *C. flavipes* are sufficient to justify disease management in parasitoid mass-rearings (Simões et al. 2012).

25.9.8 *Cotesia glomerata* (Formerly *Apanteles glomeratus*) (Hymenoptera: Braconidae)

The microsporidium *Perezia mesnili* was first reported from the imported cabbage worm *Pieris rapae* in Hawaii in 1953 (Tanada 1953). The braconid endoparasitoid *Cotesia glomerata* (formerly *Apanteles glomeratus*) also parasitizes *P. rapae* in Hawaii, where they are occasionally infected by the microsporidium (Tanada 1953, 1955). In 1966, Issi and Maslennikova report a microsporidium, *Nosema polyvora*, that develops in both the cabbage white, *Pieris brassicae*, and its parasitoid Cotesia *glomerata* from the Leningrad province.

Four species of microsporidia were subsequently described from *P. brassicae* by Paillot: *Perezia mesnili*, *P. legeri*, *P. pieris*, and *Thelohania mesnili* (see Tanada 1955). These microsporidia along with a fifth species, *Nosema polyvora*, were later recognized as the same species and combined under the name *Nosema mesnili* (see Hostounský 1970). In addition to *P. rapae* and *P. brassicae*, this microsporidium also infects the black-veined white, *Aporia crataegi* and several parasitoids, including the braconid *Cotesia* (*Apanteles*) *rubecula*; the ichneumonids *Haplaspis nanus*, *Hemiteles aerator*, *H. fulvipes*, *H. simillimus sulcatus*, *Gelis transfuga*, and *Thysiotorus brevis*; the pteromalid *Dibrachys cavus*; and the chalcid *Tetrastichus rapo* (see Hostounský 1970; Larsson 1979).

The ovaries of female *C. glomerata* that produce very few progeny contain eggs that are packed with *N. mesnili* spores. These eggs do not develop once they are inserted into the host but provide an inoculum that is capable of initiating an infection in *P. brassicae* (Issi & Maslennikova 1966). *N. mesnili* spores measure 4.0 μm (range 2.8–4.4) × 2.2 μm (1.7–3.3) (fixed and stained; Larsson 1979).

In *N. mesnili*-infected *P. rapae* larvae, the midgut epithelium and Malpighian tubules are the most heavily infected tissues (Tanada 1953). Once the microsporidium develops within the tissues, it spreads into the hemolymph. The developing parasitoid larvae are infected *per os* as they feed on host tissues. Infection of *C. glomerata* larvae first appears in the esophagus and then the silk glands, which become filled with spores. The microsporidium also infects the fat body and surrounding tissues, including the Malpighian tubules. Infection of the midgut progresses into the hindgut, and both gradually deteriorate (Hostounský 1970; Larsson 1979). Young *C. glomerata* larvae may become infected during development when the pathogen penetrates the thin wall of a spherical cavity in the larval rectum (the rectal vesicle), which is not associated with the midgut but is involved in respiration. When infected by this route, a thin layer of spores often surrounds the rectal vesicle, but the pathogen is generally not observed in any other larval tissues (Hostounský 1970; Larsson 1979). Mostly merogonic stages are observed in the rectal vesicle, and the incidence of infection is high (Larsson 1979). The gut and connective tissues are often infected in adults. Spores and vegetative stages of the pathogen infect the eggs.

Infection of *C. glomerata* by *N. mesnili* may also occur when uninfected parasitoid progeny develop within diseased *P. brassicae* larvae. Infected *C. glomerata* females transmit the pathogen to a new generation of uninfected *P. brassicae* and to the subsequent parasitoid progeny. According to Issi and Maslennikova (1966), the infected parasitoid progeny are somewhat viable, maintaining the pathogen within the parasitoid population while producing infected eggs that are able to transmit the microsporidium within the host population. However, both Hostounský (1970) and Larsson (1979) report that the pathogen is not within the parasitoid egg and host infections are initiated only when the pathogen is introduced through a contaminated parasitoid ovipositor or when it is on the egg surface.

Cotesia glomerata are able to develop in *P. rapae* with light to moderate infections but are unable to complete development in heavily infected hosts. Parasitoid larvae, pupae, and adults from heavily infected hosts contain microsporidian spores, and about one-third of the *C. glomeratus* adults that emerge from moderately infected *P. rapae* are infected with the pathogen (Tanada 1955). Larvae become infected during their final instars, and parasitoids die before they develop fully (Hostounský 1970). Infection of adults that emerge from infected *P. rapae* is dependent upon the intensity of infection in the host and the duration of contact with the pathogen. A large proportion of parasitoid larvae die as a result of the accumulation of large numbers of microsporidian spores within the gut, a lack of food reserves necessary to complete metamorphosis, and from the infection of opportunistic bacteria (Hostounský 1970). Female *C. glomerata* with heavy infections produce very few progeny (Issi & Maslennikova 1966).

In *P. rapae*, the ovaries of gravid females are infected with the microsporidium (Tanada 1955), but the male reproductive organs are not (Hostounský 1970). About one-quarter of the eggs produced by infected female *P. rapae* are infected with microsporidian spores, suggesting that the pathogen is transmitted vertically (Tanada 1955). According to Hostounský (1970), *N. mesnili* develops primarily in the gut of *P. brassicae* larvae and infects the fat body and other tissues much later. This supports the observation that *C. glomerata* larvae become infected only during their final instars. In contrast, Larsson (1979) observed the pathogen only in the fat body and silk glands but not in the gut epithelium or gut lumen. In *P. brassicae*

larvae infected by *N. mesnili*, the pathogen does not infect the digestive tract, and as a result, horizontal transmission of the pathogen by *P. brassicae* larvae does not occur.

In an experiment to examine transmission of *N. mesnili* in both *P. brassicae* and *C. glomerata*, Issi and Maslennikova (1966) administered the pathogen *per os* (4000–5000 spores per caterpillar) to second-instar *P. brassicae* larvae. As a result, all *P. brassicae* became heavily infected with the pathogen, but in *C. glomerata*, infections varied from light to heavy. When infected, first-instar *P. brassicae* larvae are parasitized by uninfected *C. glomerata*; the former is only lightly infected, and about 60% of parasitoids are infected. This agrees with prevalence data from heavily infected *P. brassicae* in the field whereby infection of *C. glomerata* does not exceed 50% and infection prevalence in the parasitoid population tends to parallel that which is observed in the host (Issi & Maslennikova 1966).

25.9.9 *Cotesia* (Formerly *Apanteles*) *marginiventris* (Hymenoptera: Braconidae)

Cotesia marginiventris is a solitary, larval endoparasitoid of noctuid larvae with a specific attraction to those of the genus *Spodoptera*. *C. marginiventris* prefers to oviposit in minute, early-instar host larvae. Prior to pupation, mature parasitoid larvae exit the host and move away from the host cadaver. When superparasitism occurs, only one parasitoid larva per host will complete its development.

Laigo and Tamashiro (1967) examined the effects of a microsporidium (*Nosema* sp.) on *C. marginiventris*. The pathogen studied is highly infectious to the lawn armyworm, *Spodoptera mauritia acronyctoides*, and is transmitted both horizontally (*per os*) and vertically (transovarially) by infected armyworm adults.

Nosema sp.-infected hosts have an adverse effect on the development of *C. marginiventris* larvae. Parasitoids that develop in infected hosts exhibit high larval and pupal mortality and reduced adult emergence. Infection is particularly detrimental to the survival of larvae during early stages in their development. The few adults that do emerge are smaller and significantly shorter-lived than those that develop in uninfected hosts. These effects are not caused by the direct infection of *Nosema* sp. in *C. marginiventris*, but rather are due to the infected host providing an inadequate environment for successful parasitoid development (Laigo & Tamashiro 1967).

The pathogen has no observable effect on the tissues of parasitoids that are reared in infected *S. mauritia acronyctoides* hosts. Adult parasitoids do not have a sufficient amount of spores to infect other hosts unless they come in contact with other *Nosema*-infected host larvae during oviposition. When this occurs, the parasitoid transmits the pathogen successfully from heavily infected to uninfected ones. Although *C. marginiventris* prefer small *S. mauritia acronyctoides* larvae, and *Nosema*-infected lawn armyworm larvae are generally smaller than uninfected ones, *C. marginiventris* do not show an ovipositional preference for *Nosema*-infected lawn armyworm hosts over uninfected ones (Laigo & Tamashiro 1967).

Several species of *Vairimorpha* infect the corn earworm, *Helicoverpa zea*, and other lepidopteran pests, and these pest species are parasitized by *C. marginiventris*. Hamm et al. (1983) studied the effects of *Vairimorpha* sp. on *C. marginiventris* when reared in infected earworm larvae. Two-day-old *H. zea* larvae, preferred hosts of the parasitoid, were fed diet that contained *Vairimorpha* sp. spores (1.6×10^5 spores/ml). Larval and pupal development of *C. marginiventris* in microsporidia-infected *H. zea* larvae are not affected, and the pathogen has no effect on adult longevity. The authors concluded that *Vairimorpha* sp. could be used for biological control of corn earworm in combination with *C. marginiventris* because the pathogen does not reduce parasitoid efficacy.

25.9.10 *Dahlbominus fuscipennis* (Hymenoptera: Eulophidae)

The microsporidium *Thelohania pristiphorae* Smirnoff infects the larch sawfly, *Pristiphora erichsonii* and other related sawfly species. The effects of this pathogen on the pupal parasitoid *Dahlbominus fuscipennis* was investigated in pupae of *P. erichsonii* and three other sawflies, including *Neodiprion swainei*, *N. erichsonii*, and *N. pratti banksianae*. Parasitoid larvae become infected with *T. pristiphorae* when they feed on microsporidia-infected sawfly pupae. The pathogen infects several parasitoid tissues, including the gut epithelia, fat body, and cells of the cerebral ganglia. *D. fuscipennis* becomes infected only in nondiapausing sawfly hosts or in those that have undergone winter diapause (Smirnoff 1971).

25.9.11 *Diadegma semiclausum* (Hymenoptera: Ichneumonidae)

The microsporidium *Vairimorpha imperfecta* infects the diamondback moth, *Plutella xylostella*, causing problems in laboratory mass-rearings. Idris et al. (2001) suggest that the *Diadegma semiclausum*, a larval parasitoid of the diamondback moth, plays a role in pathogen transmission among its hosts.

V. imperfecta spores are found on the bodies of adult parasitoids of both sexes, suggesting that adult parasitoids become contaminated with microsporidian spores during eclosion. Spores are also observed within the bodies of parasitoid adults and within the sex organs of adult females. This suggests that vertical transmission likely occurs during oviposition.

Transmission of the pathogen by *D. semiclausum* is associated with high host mortality. Many infected parasitoid adults that emerge successfully have deformed wings and are smaller than uninfected parasitoids (Idris et al. 2001).

25.9.12 *Encarsia* nr. *pergandiella* (Hymenoptera: Aphelinidae)

Encarsia nr. *pergandiella* is an endoparasitoid of the silverleaf whitefly, *Bemesia argentifolii*. A microsporidium (*Nosema* sp.) infects the ovaries of *E.* nr. *pergandiella*, resulting in a steady decline in fecundity and, ultimately, a decrease in the mass production of these wasps. Sheetz et al. (1997) treated *Nosema* sp.-infected wasps with the antibiotic rifampicin in honey solution and note a reduction in the intensity of infection. However, their data shows that infection prevalence actually increased following treatment, from an initial infection of 22.2% (having either heavy or light infections) to 30% prevalence in progeny (all having light infections). In comparison, pathogen prevalence in their control group increased from 33.3 (heavily infected) to 100% in the wasp progeny (all progeny had acquired a light infection). Sample sizes were very small and data were not analyzed. The authors speculate that the pathogen is transmitted vertically either inside the egg or on its surface.

25.9.13 *Glyptapanteles liparidis* (Hymenoptera: Braconidae)

The gregarious endoparasitoid *Glyptapanteles liparidis* is an important natural enemy of the gypsy moth, *Lymantria dispar*. Although *G. liparidis* parasitizes fourth-instar *L. dispar* larvae, the parasitoid prefers younger host larvae for oviposition (see Hoch et al. 2000).

Vairimorpha disparis (=*Vairimorpha* sp., see Vávra et al. 2006) is often isolated from *L. dispar* in Eastern Europe. Infection in *L. dispar* begins in the midgut muscles and subsequently spreads to the hemolymph, then to target tissues that include the fat body and silk glands (Solter & Maddox 1998b). When *V. disparis*-infected hosts are parasitized by *G. liparidis*, the developing parasitoid larvae are not infected systemically by the pathogen; however, parasitoid larvae ingest microsporidian spores from the host during the latter days of development. These undigested and ungerminated spores accumulate in the larval blind midgut and are excreted along with the meconium during eclosion. As a result, *G. liparidis* that develop within *V. disparis*-infected *L. dispar* larvae do not transmit the pathogen to other uninfected hosts. *G. liparidis* females do not discriminate between uninfected and *V. disparis*-infected *L. dispar* larval hosts for oviposition (Hoch et al. 2000).

Development in *V. disparis*-infected hosts prolongs the development of *G. liparidis* larvae. Adult eclosion is reduced, and those adults that successfully emerge from infected hosts are smaller, weigh less, and do not live as long as those from uninfected hosts. *L. dispar* larval hosts die prematurely when they are simultaneously infected with *V. disparis* and parasitized by *G. liparidis*. This results in the death of larval parasitoids that are unable to complete their development before the host dies and is more likely to occur when parasitization takes place when the host is already infected with the pathogen. Concurrent infection with *V. disparis* and parasitism by *G. liparidis* result in earlier death of the host than does infection with the pathogen alone (Hoch et al. 2000).

Hoch et al. (2002) report that infection by *V. disparis* and parasitization by *G. liparidis* decrease hemolymph carbohydrate and fatty acids in *L. dispar* host larvae. Because *G. liparidis* feeds on host hemolymph, such changes explain, in part, the adverse effects of *V. disparis* on the growth and development of *G. liparidis* larvae.

25.9.14 *Lydella thompsoni* (Diptera: Tachinidae)

The solitary endoparasitoid *Lydella thompsoni* is an important natural enemy of the ECB, *Ostrinia nubilalis*. Between 1944 and 1955, introductions of *L. thompsoni* into the central United States resulted in effective ECB control (see Cossentine & Lewis 1988). By the 1960s, a dramatic decline in parasitism by *L. thompsoni* led to speculation that a microsporidium pathogenic to the parasitoid may have caused its disappearance in field populations of ECB (Hill et al. 1978; Lewis 1982). This assumption may have been based on the observations of York (1961), who reports an unidentified microsporidium in dead *Lydella grisescens* larvae from field-collected *O. nubilalis*.

Cossentine and Lewis (1988) studied the effects of *Nosema pyrausta* and *Nosema* sp., two species of microsporidia pathogenic to the ECB, on *L. thompsoni*. Microsporidia-infected *O. nubilalis* larvae were fed diet that contained spores (either 100 *N. pyrausta* spores or 50 *Nosema* sp. spores/mm^2 of diet surface), and *L. thompsoni* were provided uninfected and microsporidia-treated larvae as hosts.

N. pyrausta and *Nosema* sp. cause systemic infections of *O. nubilalis* larvae. Five days following parasitization, spores are not observed in either the tissues or within the blind gut of *L. thompsoni* larvae. However, spores are observed in the alimentary canals of late-instar *L. thompsoni* larvae that emerge from both *N. pyrausta*- and *Nosema* sp.-infected hosts. Larval feeding behavior explains the absence of microsporidian spores from first- and second-instar *L. thompsoni* larvae and the accumulation of spores in the alimentary canal of third instars (Cossentine & Lewis 1988). First- and second-instar

L. thompsoni larvae feed primarily on host hemolymph, whereas third-instar larvae consume most of the internal organs of the host before emergence (see Cossentine & Lewis 1988).

A majority (58%) of *O. nubilalis* larvae that are heavily infected with *N. pyrausta* die prematurely, killing the *L. thompsoni* larvae that develop within. The number of *L. thompsoni* larvae that emerge and pupate from *N. pyrausta*-infected hosts does not differ from those that emerge from uninfected hosts. Although adult parasitoids emerge successfully from *N. pyrausta*-infected *O. nubilalis* larvae, adult *L. thompsoni* are unable to eclose from *Nosema* sp.-infected hosts, suggesting that the accumulation of *Nosema* sp. spores in the larval midgut is detrimental to parasitoid development. The authors conclude that *N. pyrausta* is not a factor in the disappearance of *L. thompsoni* from field populations of the ECB in the Midwestern United States (Cossentine & Lewis 1988).

25.9.15 *Macrocentrus ancylivorus* (Hymenoptera: Braconidae)

The braconid parasitoid *Macrocentrus ancylivorus* is an important natural enemy of the oriental fruit moth, *Grapholitha molesta* (Allen & Brunson 1947). This orchard pest was first discovered in the United States in 1915 near Washington, DC, and within a few years, it became a very destructive pest of peaches by directly damaging the fruit. Chemical controls for the oriental fruit moth were unsuccessful (Finney et al. 1947).

Before 1929, *M. ancylivorus* was a known parasitoid of the oriental fruit moth only along the coast from southern Connecticut to southern Virginia where the parasitoid provided effective control of *G. molesta* larvae (Allen 1932; Finney et al. 1947). It was soon recognized that biological control of the oriental fruit moth in California could be achieved by mass-releasing *M. ancylivorus* (Finney et al. 1947). In 1943, experimental production of *M. ancylivorus* began at Riverside, California, where the parasitoid was reared on the potato tuber moth, *Gnorimoschema operculella* (Allen & Brunson 1947).

During the summer of 1944, *M. ancylivorus* adults began to display unusual signs and symptoms in mass-rearings. The ventral abdomens of symptomatic individuals were conspicuously whitish, swollen, and malformed (Allen & Brunson 1945; Allen 1954). Microscopic examination of infected tissues revealed a microsporidian pathogen with spores that measured 3.7–5.0 × 1.8–2.2 µm. Spores are abundant in mature larvae and cocoons of *M. ancylivorus*, and both sexes of adults are infected. Spores are located in the midgut and hemolymph of the adult parasitoid and are also frequently observed between the muscle strands of the legs (Allen 1954). Infected individuals have a shortened life span and are barely able to fly, and infected females produce fewer progeny than healthy ones, resulting in serious losses in the production of parasitoids (Allen & Brunson 1945, 1947; McCoy 1947; Allen 1954). The majority of infected parasitoids are unable to complete development, and percent emergence of *M. ancylivorus* in mass-rearings is reduced by as much as 65–70% (Finney et al. 1947; McCoy 1947).

Although microsporidia cause considerable loss in parasitoid vigor and some decreased productivity during parasitoid propagation, these losses are not considered a serious threat to the continuance of mass production of *M. ancylivorus* (Allen 1954). Infection of *M. ancylivorus* by the microsporidium does not result in fewer female parasitoids produced per parent female or in higher mortality of cocoons. Such losses are considered moderate and are outweighed by other factors that affect parasitoid production, such as starvation through overstocking (Allen 1954).

The microsporidium also infects all developmental stages of the potato tuber moth with spores being detected readily in half- to full-grown larvae, pupae, and adults. In *G. operculella* larvae, spores are found in the hemolymph, silk glands, Malpighian tubules, and tissues and lumen of the midgut. Although microsporidia-infected *M. ancylivorus* exhibit distinct symptoms associated with infection, infected potato tuber moth hosts display no consistent symptoms (Allen 1954). The pathogen has adverse effects on the development and reproduction of the host (McCoy 1947; Allen 1954) and is transovarially transmitted. As a result, disease incidence in *M. ancylivorus* is always correlated with disease of the host (Allen and Brunson 1947; McCoy 1947; Allen 1954). According to McCoy (1947), vertical transmission of the microsporidium by infected *M. ancylivorus* is inefficient, and as a result, the pathogen is eliminated from the parasitoid population within three generations as long as there are no infected hosts among the breeding stock. Ironically, larvae of the oriental fruit moth, the pest species for which *M. ancylivorus* is reared, are not susceptible to infection by the microsporidium, and when heavily infected *M. ancylivorus* adults are reared on oriental fruit moth larvae, none of the emerging parasitoids are infected with the pathogen.

Control of the microsporidium is achieved through the segregation of uninfected tuber moth adults and breeding them in areas free of disease (known as the Pasteur method). This process is both effective and time-consuming (Allen & Brunson 1947; McCoy 1947), and disease-free breeding stock may accidentally become infected at any time. If this occurs, control may be obtained only by building up disease-free stocks again, a process that may take several months (Allen 1954). Heat treatments of *G. operculella* eggs (immersed in 47°C water for 20 min) proved to be very successful for control, reducing infections in both the host insects and in 75–90% of the parasitoids that were reared from them (Allen & Brunson 1947; Finney et al. 1947). Heat treatments proved to be more practical for pathogen control than the Pasteur method and did not affect the viability of *G. operculella* eggs or reduce the productivity of breeding stock (McCoy 1947; Allen 1954). Dry heat sterilization (70°C) is useful for eliminating microsporidia from inanimate objects, such as breeding trays used

for rearing, and diluted formaldehyde (1 part commercial formaldehyde to 19 parts water) is an effective general disinfectant. The formaldehyde solution kills *Nosema* spores within 2 min and evaporates without leaving objectionable residues (Allen 1954).

In 1949, Steinhaus and Hughes describe two microsporidian pathogens, *Nosema destructor* and *Plistophora californica*, from the potato tuber moth propagated in the insectary. Spore dimensions of *Nosema destructor* (2.8 × 4.0 µm; Steinhaus & Hughes 1949) are similar in size to those reported earlier for the unnamed microsporidium in *M. ancylivorus* (3.7–5.0 × 1.8–2.2 µm; Allen & Brunson 1945, 1947). In contrast, spores of *Plistophora californica* are considerably smaller than those of *N. destructor* (1.0 × 2.0 µm; Steinhaus & Hughes 1949). Based on spore measurements alone, *N. destructor* is likely the same pathogen reported earlier by Allen and Brunson (1945). In addition to the potato tuberworm, *Nosema destructor* also infects a number of other lepidopteran larvae, including those of the common sulfur (*Colias eurytheme*), California oak moth (*Phryganidia californica*), monarch butterfly (*Danaus plexippus*), beet armyworm (*Laphygma* [*Spodoptera*] *exigua*), small cabbage white (*Pieris rapae*), and coddling moth (*Carpocapsa* [*Cydia*] *pomonella*). *N. destructor* also infects two hymenopteran parasitoids: *Perisierola emigrata* (Bethylidae), a parasitoid of pink bollworm (*Pectinophora gossypiella*) and *Cremastus flavoorbitalis* (Ichneumonidae), parasitoid of the ECB (*O. nubilalis*).

25.9.16 *Macrocentrus cingulum* (Hymenoptera: Braconidae)

In 1926, the polyembryonic parasitoid *Macrocentrus cingulum* (=*grandii*) was introduced into North America for biological control of the ECB, *Ostrinia nubilalis* (Andreadis 1980; Siegel et al. 1986b). Between 1944 and 1954, *M. cingulum* was released in both Connecticut and Iowa where the parasitoid became widespread and sufficiently abundant in some areas to provide adequate corn borer control (Andreadis 1982b; Lewis 1982). Following its initial release, however, *M. cingulum* numbers began to gradually decline. Although several factors could be responsible for the gradual decrease in *M. cingulum* in release areas, this observation may be explained, in part, by the susceptibility of the parasitoid to the microsporidium *Nosema pyrausta* (see Andreadis 1982b).

Nosema pyrausta produces chronic infections that are frequently panzootic in ECB populations. *N. pyrausta* is considered to be important for the regulation of corn borer populations in the United States, and pathogen introductions have been suggested for corn borer control (see Andreadis 1982b).

The Malpighian tubules are the primary site of infection of *N. pyrausta* in *O. nubilalis* (Andreadis 1982b), but the larval silk glands and adult reproductive organs are also infected. The latter reduces longevity and adult fecundity (see Cossentine & Lewis 1987). Following eclosion, *M. cingulum* larvae consume all of the host larvae except for the exoskeleton (Andreadis 1982b). As a result, the parasitoid develops systemic infections from the ingestion of spores when parasitoid larvae emerge from *N. pyrausta*-infected hosts (Andreadis 1980; Siegel et al. 1986a). Infection in the parasitoid spreads rapidly throughout the body with systemic infections developing in the midgut epithelia, fat body, muscles, nerves, and Malpighian tubule cells. Gonadal tissues remain uninfected, which may explain why *N. pyrausta*-infected female parasitoids are unable to transmit the pathogen to other corn borer hosts. However, infected *M. cingulum* females may also be unable to transmit the pathogen because they die prior to completing their minimum preovipositional period of 3–4 days (Andreadis 1980). According to Siegel et al. (1986a), infected *M. cingulum* females not only emerge and survive the 3- to 4-day preoviposition period but also transmit *N. pyrausta* to uninfected hosts. The resulting progeny are also infected and capable of transmitting the pathogen. Cossentine and Lewis (1987) report that *N. pyrausta*-infected *M. cingulum* females live up to 14 days. The parasitoids in these three studies were reared and maintained at different temperatures, and it is possible that the longevity of infected *M. cingulum* adults is shorter at higher temperatures (25°C, Andreadis 1980; 19–21°C, Siegel et al. 1986b; 16–21°C, Cossentine & Lewis 1987).

When infected with *N. pyrausta*, fewer *M. cingulum* larvae emerge from their hosts. Although *N. pyrausta* has no effect on the ability of emerging larvae to pupate successfully, larval mortality occurs after fourth-instar larvae exit their hosts. Adult emergence is also reduced, and infection shortens adult longevity, decreases parasitoid fecundity, and may also reduce the number of hosts available for parasitization. Infection with *N. pyrausta* also induces a noticeable behavioral change in *M. cingulum* larvae. Uninfected parasitoid larvae tend to cluster together prior to spinning their cocoons; however, *N. pyrausta*-infected larvae scatter, and many die before spinning their cocoons. Those that do spin cocoons have a low level of eclosion when compared to uninfected larvae. *M. cingulum* females transmit *N. pyrausta* vertically, acting as vectors for the pathogen under laboratory conditions. Infected *M. cingulum* adults may also be significant vectors of *N. pyrausta* in the field (Siegel et al. 1986a).

Mature *N. pyrausta* spores from *M. cingulum* measure 4.23 ± 0.06 µm × 1.75 ± 0.02 µm (mean ± SE; Andreadis 1980) and are larger than those of *N. destructor* reported from the braconid *M. ancylivorus* (3.7– 5.0 × 1.8–2.2 µm; Allen 1954). The prevalence of infection of *N. pyrausta* in *M. cingulum* populations closely parallels that of the ECB host population (Andreadis 1980; Siegel et al. 1986a), an observation also made for *M. ancylivorus* and the potato tuber moth (Allen & Brunson 1947; McCoy 1947; Allen 1954). Andreadis (1980) concludes that this noted parallel in infection prevalence between host and parasitoid may prevent parasitoid establishment, especially when host infections are high. *N. pyrausta* may

have a significant impact on field populations of *M. cingulum*, and this could explain the reduced numbers of this parasitoid in areas where it was once abundant (Andreadis 1982b).

Under laboratory conditions, Cossentine and Lewis (1987) evaluated the effects of *N. pyrausta*, *Nosema* sp., and *Vairimorpha necatrix* on *M. cingulum* development. Both *N. pyrausta* and *Nosema* sp. are naturally associated with ECB populations (see Cossentine & Lewis 1987), and *V. necatrix* is highly virulent to *O. nubilalis*.

Of the three microsporidia, only *Nosema pyrausta* and *Nosema* sp. infect the tissues of *M. cingulum* larvae. *Nosema pyrausta* infects the Malpighian tubules and silk glands of host larvae as well as the fat body and muscle tissues. Intensive infections in *O. nubilalis* larvae result in reduced parasitoid emergence. *Nosema* sp. infects midgut epithelial cells and the lumen of the alimentary canal. As in the case of *N. pyrausta*, the first three larval instars of *M. cingulum* are unlikely to ingest *Nosema* sp. spores during their development, but spores are ingested when the host body is consumed following emergence (Siegel et al. 1986a; Cossentine & Lewis 1987). All *M. cingulum* larvae that develop within, and emerge from, *Nosema* sp.-infected *O. nubilalis* larvae are infected with the pathogen. Spores are found in the midgut epithelium and lumen of the alimentary canal.

In *O. nubilalis* larvae, *V. necatrix* causes damage to the midgut, bacterial septicemia, and death. Larvae that survive may ultimately die as pupae (Lewis 1982). About half of the fourth-instar *M. cingulum* that emerge from *V. necatrix*-infected *O. nubilalis* larvae eclose as adults. Only the lumens of the alimentary canal of *M. cingulum* larvae are infected. Adult parasitoid eclosion is decreased by all three microsporidia (*N. pyrausta*, *Nosema* sp., and *V. necatrix*), and only male parasitoids eclose from *Nosema*-infected hosts. *M. cingulum* females infected with either *N. pyrausta* or *Vairimorpha necatrix* do not transmit the pathogen vertically (Cossentine & Lewis 1987).

25.9.17 *Muscidifurax raptor* (Hymenoptera: Pteromalidae)

The pteromalid endoparasitoid *Muscidifurax raptor* is an important natural enemy of muscoid flies in the northeastern United States (see Geden et al. 1992). Not only are houseflies, *Musca domestica*, and stable flies, *Stomoxys calcitrans*, serious insect pests in agricultural production systems, but the migration of large populations of flies from farms to neighboring urban areas is problematic for those residing there (see Dry et al. 1999).

Naturally occurring populations of *Muscidifurax raptor* are often encountered on dairy farms in the northeastern United States where they provide effective biological control of both house and stable flies. In addition, many commercial insectaries mass-produce *M. raptor* for inundative release to maintain fly populations below nuisance thresholds (see Zchori-Fein et al. 1992).

Field-collected *M. raptor* often exhibit a rapid and profound loss of fitness when reared under laboratory conditions. Geden et al. (1992) observed that both parasitization rates and progeny production of field-collected parasitoids are reduced by half after being reared for only two generations in the laboratory. Zchori-Fein et al. (1992) report that one-third of parasitoids collected from New York dairy farms in 1990 were infected with a microsporidium, later described as *Nosema muscidifuracis* (see Becnel & Geden 1994). Although microsporidia are common pathogens of parasitoids, *N. muscidifuracis* is one of only three known microsporidian pathogens that specifically infect a parasitoid and not its host (Geden et al. 1995).

N. muscidifuracis also infects insectary-reared parasitoids. In a 1995 study, 86–100% of *M. raptor* obtained from a commercial insectary for biological control were infected with *N. muscidifuracis* (Geden et al. 1995). *N. muscidifuracis* was found in 1.1 and 10.7% of indigenous *M. raptor* collected from New York dairy farms in 1991 and 1992, respectively. However, on farms that released commercially reared *M. raptor* for biological fly control, the prevalence of the microsporidium was as high as 84%. Dry et al. (1999) examined *M. raptor* biweekly from poultry houses in Arkansas and report the prevalence of microsporidia in *M. raptor* as 13 and 5% in 1994 and 1995, respectively. In contrast to the observations made by Geden et al. (1995), higher disease prevalence was not observed among parasitoids on farms where commercially reared *M. raptor* were released, and the authors conclude that released wasps are not a major source of the pathogen.

N. muscidifuracis produces two types of spores in larval and adult *M. raptor*: one spore type is distinguished by a short polar filament (with about five turns) and is thought to be involved in pathogen transmission to new host cells (autoinfection); the second has a longer polar filament (about 9–10 turns) and is likely involved in pathogen transmission to new susceptible hosts (Becnel & Geden 1994). Mature spores are diplokaryotic and ovoid and measure $5.4 \pm 0.5 \times 3.0 \pm 0.2\,\mu m$. Within the eggs of *M. raptor* are both vegetative stages and spores, in which the latter differ from those in the larvae and adults. Spores within the eggs are elongate oval and measure $6.0 \pm 0.8 \times 3.2 \pm 0.3\,\mu m$. The isofilar polar filament is coiled about 15–16 times around the posterior vacuole and characteristically lacks an obvious exospore. Because vegetative stages are found in *M. raptor* eggs, these may be able to initiate an infection in the developing progeny. It is possible that the spores within *M. raptor* eggs are not transmitted vertically but permit horizontal transmission of the pathogen to new hosts when eggs are cannibalized. Vegetative stages of the pathogen infect the midgut epithelium, Malpighian tubules, ovaries and oocytes, and fat body of both larvae and adults (Becnel & Geden 1994).

Vertical transmission of *N. muscidifuracis* is 100% efficient (Zchori-Fein et al. 1992). The maintenance of high pathogen prevalence of *N. muscidifuracis* in field populations of *M. raptor* suggests that efficient vertical transmission is also

accompanied by periodic horizontal transmission of the pathogen. Both known mechanisms of horizontal transmission involve cannibalism. Horizontal transmission occurs when uninfected *M. raptor* immatures feed on infected immatures within superparasitized host puparia or when adult parasitoids feed on the fluids of infected parasitoid immatures through wounds made by their ovipositors in the host pupae (Becnel & Geden 1994; Geden et al. 1995). Horizontal transmission of *N. muscidifuracis* increases when parasitoids are overcrowded, resulting in the cannibalism of infected immatures by healthy ones within superparasitized hosts. This increases the prevalence of *N. muscidifuracis* in as few as two generations under laboratory conditions (Geden et al. 1992). When fed suspensions of *N. muscidifuracis* obtained from macerated *M. raptor* tissue (ranging from 2.1×10^6 to 2.1×10^7 spores), horizontal transmission of the pathogen to *M. raptor* adults is 100%, and parasitoid mortality increases with an increase in dose. In contrast, housefly adults and larvae are not infected and are not involved in pathogen transmission (Zchori-Fein et al. 1992; Geden et al. 1995). The pathogen is not transmitted by paternal or venereal transmission (Geden et al. 1995).

When compared to uninfected parasitoids, those with systematic infections of *N. muscidifuracis* take longer to complete development, live half as long, and produce about one-tenth of the progeny that are normally produced by uninfected parasitoids (Zchori-Fein et al. 1992; Geden et al. 1995). These effects are profound and result in reduced reproductive success of infected parasitoids and reduced fly control (Geden et al. 1992; Zchori-Fein et al. 1992; Dry et al. 1999).

Uninfected *M. raptor* colonies may be established through the isolation of uninfected parasitoids from those infected with the pathogen (Pasteur method) and using the progeny of the former to establish uninfected colonies (Zchori-Fein et al. 1992). The development of infected *M. raptor* is about 7% longer than that of uninfected parasitoids, resulting in developmental differences that provide narrow windows for identifying and isolating uninfected *M. raptor* females from infected ones when establishing uninfected colonies (Boohene et al. 2003a).

Heat treatments are also successful for eliminating disease from established laboratory colonies of *M. raptor* that are infected with *N. muscidifuracis.* The immersion of infected *M. raptor* eggs within fly puparia in a water bath (30–60 min at 47°C) reduces pathogen prevalence. The elimination of the pathogen from an established *M. raptor* colony results in an increase in parasitoid fecundity. Exposures ranging from 30 to 45 min are highly effective for reducing disease prevalence. When parasitoid eggs within host puparia are subjected to prolonged heat treatment (45°C for 5 h), only uninfected adult parasitoids emerge (Boohene et al. 2003b). However, lengthy exposure to heat treatments may reduce parasitoid survival by causing high host mortality (Geden et al. 1995). Although heat treatments are effective at reducing pathogen prevalence, the procedure does not eradicate the pathogen. For colonies where all individuals are infected with *N. muscidifuracis*, it may be practical to use heat treatments to reduce disease prevalence, then use the Pasteur method to isolate uninfected parent wasps, and rear progeny from them (Geden et al. 1995).

The continuous rearing of wasps at elevated temperatures (30–32°C) results in decreased spore loads but does not eliminate the pathogen (Boohene et al. 2003b).

Albendazole and fumagillin treatments have no effect on pathogen prevalence in *M. raptor* adults or their progeny, but treatment decreases the rate of transovarial transmission (Geden et al. 1995; Boohene et al. 2003b). Treatment of *N. muscidifuracis*-infected *M. raptor* eggs or pupae with gamma radiation is ineffective as a control (Boohene et al. 2003b).

The release of microsporidia-infected parasitoids for biological control may result in the introduction of the pathogen into field populations where it is otherwise rare. Geden et al. (1995) recommends that natural enemies be subject to rigorous quality control to ensure that only uninfected *M. raptor* are used for biological pest control.

25.9.18 *Pediobius foveolatus* (Hymenoptera: Eulophidae)

Pediobius foveolatus parasitizes the larvae of the Mexican bean beetle, *Epilachna varivestis*. In 1980, two microsporidia from *E. varivestis* were described as *Nosema epilachnae* and *N. varivestis* (Brooks et al. 1985).

Infections of *N. epilachnae* in the Mexican bean beetle are systemic, but the adipose tissue, muscles, and Malpighian tubules of larvae become heavily infected. The pathogen also infects the adult reproductive tissues. The pathogen is highly virulent, and larvae and adults are infected *per os*, and the pathogen is transmitted transovarially. Diplokaryotic spores are ovocylindrical in shape and measure $5.3 \pm 0.13 \times 2.1 \pm 0.03$ µm (Brooks et al. 1980, 1985).

Pediobius foveolatus that develop within beetle larvae infected with *N. epilachnae* as first instars are highly susceptible to the pathogen. The majority of *P. foveolatus* progeny (96%) become infected with the pathogen during development. Few of these progeny are able to complete development, and parasitoid mortality occurs primarily in the pupal stage during eclosion. As a result, fewer parasitoid progeny are produced from *N. epilachnae*-infected hosts than from healthy ones. Furthermore, heavily infected *P. foveolatus* females produce fewer progeny per host, and infection among these progeny is 100% (Own & Brooks 1986).

The duration of parasitoid development is unaffected by the pathogen; however, longevity is significantly reduced. About one-third (34%) of adult parasitoids infected with *N. epilachnae* have malformed wings, greatly distended abdomens, or both. A small percentage of parasitoids escape infection and successfully eclose as adults. Mechanical transmission of the pathogen from infected parasitoids to uninfected hosts occurs during oviposition (Own & Brooks 1986).

The pathogen *Nosema varivestis* also results in systemic infection of the host. The Malpighian tubules of *E. epilachnae* larvae are heavily infected, and the adult reproductive tissues are also invaded by the pathogen. The midgut epithelium remains uninfected. Diplokaryotic spores are ellipsoidal to slightly pyriform and measure $4.7 \pm 0.06 \times 2.6 \pm 0.03$ µm. Virulence of *N. varivestis* in the host is low (Brooks et al. 1980, 1985).

P. foveolatus is also highly susceptible to *N. varivestis*, particularly when late-instar hosts that were infected as first-instar larvae are parasitized. Under such conditions, all of the progeny are infected, and only about half are able to emerge successfully from *N. varivestis*-infected bean beetle larvae. Mean progeny production is less in *N. varivestis*-infected hosts than in uninfected ones. As is the case with *N. epilachnae*, some *P. foveolatus* adults infected with *N. varivestis* have malformed wings and greatly swollen abdomens (Own & Brooks 1986).

Infection in *P. foveolatus* is systemic, and spores are observed in the adipose tissue, midgut epithelial cells, muscles, and ventral nerve cord. The pathogen has no effect on the duration of larval development but significantly reduces adult longevity. Adult *P. foveolatus* are infected *per os*. Vertical transmission from infected wasp adult to its progeny is low (about 29%) as is mechanical transmission of the pathogen during oviposition (Own & Brooks 1986).

Chapman and Hooker (1992) report *Nosema* sp. in the fat body, midgut muscle, gut epithelium, hemocoel, Malpighian tubules, and eggs of *P. foveolatus.* Diplokaryotic spores are ovocylindrical and measure 4.5×2.0 µm. Based on spore size and the host tissues that are infected, the authors conclude that the pathogen is *N. varivestis.* However, infected wasps showed no outward signs of infection.

The susceptibility and detrimental effects of both *N. epilachnae* and *N. varivestis* emphasize the importance of mass-rearing *P. foveolatus* in uninfected *E. epilachnae* larvae. Undetected infections may reduce the efficacy of *P. foveolatus* used in biological control programs (Own & Brooks 1986; Chapman & Hooker 1992).

25.9.19 *Tachinaephagus zealandicus* (Hymenoptera: Encyrtidae)

Tachinaephagus zealandicus is a gregarious parasitoid of third-instar muscoid fly larvae. Field-collected *T. zealandicus* from Brazil were infected with an undescribed microsporidium. All stages of the parasitoid are susceptible to infection (Geden et al. 2002). Diplokaryotic spores (*Nosema* sp.) measure $4.16 \pm 0.12 \times 2.05 \pm 0.07$ µm and are observed in the gut, Malpighian tubules, fat body, ovaries, and muscle of adult parasitoids. Few spores (<10 spores/egg) are observed within newly deposited *T. zealandicus* eggs. The pathogen does not infect the flesh fly, *Sarcophaga bullata*, and the Old World screwworm fly, *Chrysomya putoria*, two *T. zealandicus* hosts (Ferreira de Almeida et al. 2002).

Vertical transmission of the pathogen is 96.3%. Infection of newly emerged *T. zealandicus* progeny is partially controlled (63%) when adult females are fed rifampicin (bactericidal antibiotic) mixed with honey as food (ad libitum, minimum of 8 days). In contrast, all of the progeny produced rifampicin- and albendazole (anthelmintic)-treated parasitoids are infected. Uninfected *T. zealandicus* immatures become infected when they share superparasitized hosts with microsporidia-infected immatures, but the mechanisms of transmission are unknown (Ferreira de Almeida et al. 2002).

When fed a diet of honey and water, *Nosema* sp.-infected male and female parasitoids live about half as long as do uninfected females. This reduction is most pronounced when parasitoids are reared at 15 or 30°C. The microsporidium does not affect the duration of development of immature parasitoids, but adult emergence is reduced significantly. Many infected parasitoids develop fully to the adult stage but fail to emerge from host puparia. Sex ratios of infected parasitoids are more male-biased when compared to uninfected parasitoids (Geden et al. 2002).

Nosema sp.-infected *T. zealandicus* parasitize fewer housefly (*Musca domestica*) larvae and produce less than half the number of progeny that are produced by uninfected wasps. When provided *S. bullata* as hosts, infected wasps kill as many hosts and produce as many progeny as do uninfected wasps. When provided with a continual supply of honey, water, and *M. domestica* larval hosts, infected parasitoids live as long as uninfected ones, but the latter attack a significantly greater number of hosts and produce twice as many adult progeny (Geden et al. 2002).

25.9.20 *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae)

The parasitoid *Trichogramma chilonis* prefers to parasitize eggs of the cabbage moth, *Plutella xylostella*, and is therefore considered to be a promising candidate for biological control of this lepidopteran pest (see Schuld et al. 1999). Infection with *Vairimorpha* sp. has little impact on the fitness of *P. xylostella* in laboratory mass-rearings. However, *Vairimorpha* sp. also infects *T. chilonis* when infected *P. xylostella* eggs are parasitized and the fitness of parasitoid progeny is adversely affected (Schuld et al. 1999).

T. chilonis larvae ingest the pathogen when they feed on the host egg, and *Vairimorpha* sp. spores are found in the intestinal lumen of *T. chilonis* larvae 3 days following parasitization. The pathogen develops in several *T. chilonis* tissues, including the flight muscle and the nervous system. Infection results in reduced adult emergence, longevity, and fecundity. Parasitoid emergence is related to host age: significantly fewer adults eclose from 24 to 72 h-old *Vairimorpha* sp.-infected eggs than from eggs that are less than 24 h old. Parasitization efficacy of *T. chilonis* offspring is also reduced in a host

age-related pattern. Infection with *Vairimorpha* sp. has no effect on the sex ratio of emerging *T. chilonis* or the duration of parasitoid development, and there is no evidence to suggest that *Vairimorpha* sp. is transmitted vertically by *T. chilonis*. The authors conclude that if *T. chilonis* is to be used for biological control of *P. xylostella*, the parasitoid must be reared on pathogen-free host eggs to ensure that progeny are viable and effective (Schuld et al. 1999).

25.9.21 *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae)

The microsporidium *Nosema pyrausta* causes chronic infections in the ECB, *Ostrinia nubilalis*. The pathogen is vertically transmitted within *O. nubilalis* populations. The lepidopteran egg parasitoid *Trichogramma evanescens* parasitizes both uninfected and *N. pyrausta*-infected *O. nubilalis* eggs, and parasitoid development and emergence from uninfected and infected hosts are similar. However, parasitoid progeny that emerge from *N. pyrausta*-infected hosts are susceptible to infection (Huger 1984).

In heavily infected parasitoid progeny, the pathogen is observed in several tissues, including the alimentary tract, fat body, Malpighian tubules, muscles, nervous system, and hypodermis. In some cases, the abdomen is filled with spores. When *N. pyrausta*-infected *T. evanescens* are provided eggs from the Angoumois grain moth *Sitotroga cerealella* for oviposition, mean fecundity is reduced by half. *T. evanescens* do not transmit the pathogen vertically (Huger 1984).

25.9.22 *Trichogramma nubilale* (Hymenoptera: Trichogrammatidae)

Trichogramma nubilale shows a preference for eggs of the ECB, *Ostrinia nubilalis* (see Sajap & Lewis 1988). The microsporidium *N. pyrausta* infects *O. nubilalis* and several of its parasitoids, including *T. nubilale* (York 1961; Andreadis 1980; Huger 1984; Siegel et al. 1986b; Cossentine & Lewis 1987; Cossentine & Lewis 1988; Sajap & Lewis 1988).

Infection of *T. nubilale* occurs when adult females oviposit in infected host eggs. *N. pyrausta* spores are ingested during larval development and accumulate within the lumen of the alimentary tract, reducing the area that is used for food storage. In pupae and adults, the pathogen infects tissues adjacent to the gut epithelium, muscles, and neural tissues. Progeny produced by infected *T. nubilale* females are not infected with the pathogen, and as a result, *T. nubilale* do not transmit *N. pyrausta* vertically (Sajap & Lewis 1988).

The duration of parasitoid development, adult longevity, and adult sex ratios are not affected by the pathogen. However, adult emergence is reduced significantly when *T. nubilale* develop in *N. pyrausta*-infected eggs, and infection also results in reduced fecundity (Sajap & Lewis 1988; Saleh et al. 1995). *T. nubilale* progeny that eclose from *N. pyrausta*-infected *O. nubilalis* eggs are smaller and weigh less than those that emerge from uninfected eggs. This reduction in the size and weight of *T. nubilale* adults suggests that *N. pyrausta*-infected *O. nubilalis* eggs are less suitable for parasitoid development than are uninfected host eggs (Saleh et al. 1995). When *T. nubilale* are mass-reared for biological control of the ECB, it is important that they be reared on eggs free of *N. pyrausta* (Sajap & Lewis 1988).

T. nubilale females parasitize both *N. pyrausta*-infected and uninfected eggs, showing no preference for uninfected hosts over those that are infected (Sajap & Lewis 1988; Saleh et al. 1995). This lack of host discrimination may enhance *T. nubilale* efficacy because many field populations of *O. nubilalis* are infected with *N. pyrausta* (Andreadis 1986).

25.10 PHYTOPHAGOUS INSECTS

25.10.1 *Galeruca rufa* (Coleoptera: Chrysomelidae)

In the 1980s, the chrysomelid *Galeruca rufa* was considered a potential candidate for the control of field bindweed, *Convolvulus arvensis.* Beetles could severely defoliate plants in field cages, but later, they were found to also feed upon several varieties of sweet potato. As a result, despite their efficacious control of field bindweed, *G. rufa* were never released in the United States (Rosenthal 1995).

During preliminary testing, the prevalence of *Nosema* sp. in *G. rufa* imported from Italy was between 61 and 72%. Healthy females produced significantly more eggs than did infected females, but the pathogen had no effect on egg hatch. *Nosema* sp. is easily eliminated from *G. rufa* reared in the laboratory. Most infected beetles produce uninfected progeny, and these may be isolated to ensure disease-free colonies. Horizontal transmission of the pathogen is likely due to contamination of the eggs or rearing materials by infected feces, which contain large concentrations of microsporidian spores (Etzel et al. 1981).

Etzel et al. (1981) report microsporidioses in several other species studied for their potential as weed control agents. At the time, 5 of 17 beetle species were infected with *Nosema*. The authors comment that microsporidia likely infect other beetle species and would be reported with higher frequency if only there was a reason for these beetle species to be examined. The debilitating effects of microsporidia are usually sufficient to ensure that potential biological control candidates be confirmed microsporidia-free prior to their evaluation and release.

25.10.2 *Lema cyanella* (Coleoptera: Chrysomelidae)

In the late 1970s, the chrysomelid *Lema cyanella* was being evaluated for its biological control potential against Canada thistle, *Cirsium arvense*, in Canada. A colony of field-collected beetles from Germany was infected with *Nosema* sp., and the pathogen gradually destroyed a laboratory colony of this promising weed control candidate. The pathogen has no effect on adult longevity, but infection causes beetles to cease copulation and oviposition. Mortality among eggs and larvae is high, and progeny reared to the third generation have a highly skewed female sex ratio (4 males to 84 females), but none of these females develop ovaries. Based on these observations, the authors recommend that only disease-free beetles be released in North America (Peschken & Johnson 1979). In 1983, *Lema cyanella* was approved for release in Canada (McClay et al. 2001).

25.10.3 *Rhinocyllus conicus* (Coleoptera: Curculionidae)

The weevil *Rhinocyllus conicus* was introduced to California from Italy for biological control of Italian thistle, *Carduus tenuiflorus*. Infection of *R. conicus* with *Nosema* sp. led to an awareness of the presence of pathogens in insect candidates for the biological control of weeds. Dunn and Andres (1980) found microsporidia (*Nosema* sp.) infecting the crown weevil, *Ceutorhynchus litura*, and the Canada thistle stem gallfly, *Urophora cardui*, two biological control agents that had been released for biological control of Canada thistle, *Cirsium arvense*. The authors also report *Nosema* sp. infections in the chrysomelids *Aphthona flava* and *A. nigriscutis* (biological control candidates for leafy spurge, *Euphorbia esula*), *Galeruca rufa* (candidate for control of field bindweed, *Convolvulus arvensis*), and *Lema cyanella* (a candidate for controlling Canada thistle). The weevil *Ceutorhynchus trimaculatus*, a candidate for control of nodding thistle, *Carduus nutans*, was also infected with *Nosema* sp.

25.11 PREDATORS

25.11.1 *Adalia bipunctata* (Coleoptera: Coccinellidae)

Two-spotted lady beetles, *Adalia bipunctata*, are commercially available for aphid control in North America and Europe. The microsporidium *Nosema adaliae* has recently been described from field-collected *A. bipunctata* (Steele & Bjørnson 2014), and because lady beetles are known to be susceptible to more than one species of microsporidia, Steele and Bjørnson (2012) examined the effects of two microsporidian pathogens on the fitness of *A. bipunctata* under laboratory conditions.

When infected with N. adaliae, the development of *A. bipunctata* larvae is prolonged significantly; however, the microsporidium *Tubulinosema hippodamiae* (from the convergent lady beetle, *Hippodamia convergens*) has no effect on larval development. When infected with both pathogens, larval development is also prolonged significantly, but this observation may be caused solely by *N. adaliae* and not from a combined effect of the two. Infection with one or both microsporidia did not affect sex ratios, adult fecundity, or longevity. Although N. adaliae spores ($5.43 \pm 0.06 \times 2.75 \pm 0.03\,\mu m$) are larger than those of *T. hippodamiae*, spores of these microsporidia are difficult to differentiate when specimens infected with both pathogens are examined by light microscopy (Steele & Bjørnson 2012).

25.11.2 *Chrysoperla* (Formerly *Chrysopa*) *californica* (Neuroptera: Chrysopidae)

Albert Koebele first noted the potential of *Chrysoperla* for biological pest control in 1888 during his trip to Australia in search of natural enemies of the cottony cushion scale, *Icerya purchasi* (see Koebele 1890). Today, *Chrysoperla carnea* and *C. rufilabris* are mass-produced for aphid control in Europe and North America (van Lenteren et al. 1997).

In 1949, the microsporidium *Pleistophora* (=*Plistophora*) *californica*, a pathogen of the potato tuberworm, *Gnorimoschema operculella*, was noted to infect several insect hosts, including *Chrysoperla californica* (see Steinhaus & Hughes 1949). *P. californica* had also become a significant pest in mealybug colonies that were used as food for rearing the predatory coccinellid, *Cryptolaemus montrouzieri* (see Finney et al. 1947). Although *C. californica* is a nontarget host of *P. californica*, this pathogen reduces lacewing longevity and fecundity (Finney 1950). *P. californica* spores ($1.0 \times 2.0\,\mu m$; Steinhaus & Hughes 1949) are made unviable by immersing infected host eggs and larvae in a hot water bath (5 min at 135°C) before providing them as food for *Chrysoperla* larvae (Finney 1950).

Sajap and Lewis (1989) examined the effects of *Nosema pyrausta*, a pathogen of the ECB, *Ostrinia nubilalis*, on *Chrysoperla carnea*. The pathogen had no effect on the fecundity or longevity of *C. carnea*. Ingested spores remain confined to the lumen of the larval blind midgut throughout development and are voided in the meconium during eclosion. As a result, these spores do not infect the adult tissues, but they do remain infective to *O. nubilalis* larvae (Sajap & Lewis 1989).

25.11.3 *Coccinella septempunctata* (Coleoptera: Coccinellidae)

Adult seven-spotted lady beetles, *Coccinella septempunctata*, fed a mixture of honey and *Nosema tracheophila* spores become infected. The pathogen is also transmitted when adult beetles are fed the bodies of dead, infected beetles. Infected adults do not exhibit any obvious signs or symptoms. Vegetative stages of pathogen are observed in the hemocytes. The oval spores (4.0–5.3 × 2.2 × 3.1 µm) infect the tracheal epithelium, the connective tissue that surrounds the fat body, Malpighian tubules, gonads, and midgut musculature (Cali & Briggs 1967).

Nosema coccinellae infects field-collected *C. septempunctata*, *H. tredecimpunctata* (13-spotted lady beetle), and *Myrrha octodecimguttata* (18-spotted lady beetle) from Poland and Russia. Pathogen prevalence in these beetles was 24.1, 8.7, and 2.5%, respectively (Lipa & Semyanov 1967; Lipa 1968). Lipa et al. (1975) later report *N. coccinellae* in *Adalia bipunctata* (two-spotted lady beetle), *Coccinella quinquepunctata* (five-spotted lady beetle), and *Exochomus quadripustulatus* (pine lady beetle).

Ellipsoidal spores measure 4.4–6.7 × 2.3–3.4 µm (fresh) and 3.6–6.2 × 2.0–3.6 µm (fixed and stained) and are larger than those of *N. hippodamiae* reported from the convergent lady beetle (*Hippodamia convergens*). The pathogen produces localized infections in cells of the midgut epithelium, Malpighian tubules, gonads, nerves, ovaries, and muscles. In *C. septempunctata*, the Malpighian tubules are the most heavily infected tissue, becoming greatly distended. Tissue pathology is less pronounced in the midgut. In *M. octodecimguttata*, the gut is the most severely infected tissue and is frequently destroyed by the pathogen. The ovaries and oocytes are also infected (Lipa & Semyanov 1967; Lipa 1968).

25.11.4 *Hippodamia convergens* (Coleoptera: Coccinellidae)

Convergent lady beetles, *Hippodamia convergens*, are commercially available for aphid control in home gardens and in agricultural cropping systems throughout the United States and Canada. Beetles are collected annually from the Sierra Nevada Mountains of California for redistribution, a practice that has occurred for more than 100 years (Carnes 1912).

In 1959, Lipa and Steinhaus reported a microsporidium in *H. convergens* collected in California. Diseased beetles do not show external signs or symptoms. The pathogen, *Nosema hippodamiae*, has ovoid spores that measure 3.3–5.4 × 2.2–2.7 µm. The primary site of infection is the midgut and fat body, but the pathogen is observed in other tissues when the host is heavily infected.

A second microsporidium, *Tubulinosema hippodamiae*, was described from *H. convergens* obtained from a commercial insectary for aphid control. The life cycle and pathology of *T. hippodamiae* bear some similarities to that of *N. hippodamiae*, reported earlier by Lipa and Steinhaus (1959). Diplokaryotic spores measure 3.58 ± 0.2 × 2.06 ± 0.2 µm and have a polar filament with 10–14 coils arranged in a single or double row. Aberrant spores are also observed. These measure 3.38 ± 0.8 × 2.13 ± 0.2 µm and have a fully developed spore wall but lack any internal spore structures. Instead, the interior is filled with lamellar or vesicular structures. The pathogen infects longitudinal muscle that surrounds the midgut, and spores are observed in several tissues, including the fat body, Malpighian tubules, hindgut and pyloric valve epithelia, ventral nerve cord ganglia, muscles, and ovaries. The connective tissues are rarely invaded (Bjørnson et al. 2011).

T. hippodamiae was detected in individuals from 13 to 22 shipments of adult *H. convergens* from three commercial insectaries. Although the prevalence of *T. hippodamiae* in commercially available beetles is low (0.9%; range 0–3%), *H. convergens* are sold and released in large numbers, and it is likely that thousands of these beetles are infected with microsporidia (Bjørnson 2008).

T. hippodamiae prolongs the development of *H. convergens* larvae and reduces the fecundity and survival of adults. The pathogen is transmitted horizontally with 100% efficiency among *H. convergens* cohorts and other coccinellids when microsporidia-infected *H. convergens* eggs are cannibalized (Saito & Bjørnson 2006, 2008; Joudrey & Bjørnson 2007). Under laboratory conditions, *T. hippodamiae* infects several coccinellids, including *Adalia bipunctata* (the two-spotted lady beetle), *Coccinella septempunctata* (seven-spotted lady beetle), *C. trifasciata perplexa* (three-banded lady beetle), and *Harmonia axyridis* (multicolored Asian lady beetle). For all of these beetle species, the development of *T. hippodamiae*-infected larvae is prolonged when compared to uninfected larvae, but the pathogen has no effect on larval mortality (Saito & Bjørnson 2006, 2008).

Mean spore counts from smear preparations of infected beetles indicate that infection is as heavy in *A. bipunctata* and *C. trifasciata perplexa* (native coccinellids) as it is in *H. convergens* (the natural host), but infection is lighter in both *C. septempunctata* and *H. axyridis* (introduced species). This suggests that the two native coccinellids are more suitable as hosts for the pathogen than is either of the introduced species. Although *T. hippodamiae*-infected *H. convergens* produce fewer eggs and do not live as long as uninfected beetles, the pathogen has no effect on the fecundity and longevity of *A. bipunctata*, *C. septempunctata*, or *H. axyridis*. Vertical transmission of the pathogen reached 100% during a 90-day ovipositional study when these beetles were fed *T. hippodamiae*-infected eggs as larvae (Saito & Bjørnson 2008).

The braconid *Dinocampus coccinellae*, an endoparasitoid of several coccinellid species, becomes infected with *T. hippodamiae* when wasp larvae develop within microsporidia-infected *H. convergens* hosts. Although horizontal

transmission from beetles to wasp progeny is 100%, the pathogen has no effect on larval development. A greater proportion of beetles stung by uninfected wasps contained an endoparasitoid larva, suggesting that infection reduces wasp fecundity or egg viability. The pathogen infects all *D. coccinellae* adult tissues with exception of the ovary. Adult wasps do not show a preference for either uninfected or microsporidia-infected *H. convergens* as hosts (Saito & Bjørnson 2013).

25.11.5 *Metaseiulus occidentalis* (Acari: Phytoseiidae)

The predatory mite, *Metaseiulus occidentalis*, is mass-reared in commercial insectaries for spider mite control on various horticultural and agricultural crops. Laboratory-reared colonies of *M. occidentalis* are susceptible to the microsporidium *Oligosporidium occidentalis*. The haplokaryotic spores of this pathogen have two distinct morphologies. The first spore type, most common in nymphs and young adults, measures $2.53 \times 1.68\,\mu m$, has a short polar filament (three to five coils), and is thought to be involved in autoinfection and vertical transmission. The second type of spore, observed in older adult mites, measures $3.14 \times 1.77\,\mu m$, has a longer polar filament (eight to nine coils), and may be responsible for horizontal transmission of the pathogen when infected eggs are cannibalized (Becnel et al. 2002).

The pathogen infects all developmental stages of the mite (eggs, larvae, nymphs, and adults), but mites infected with the pathogen do not show any signs of infection. *O. occidentalis* developmental stages and spores are observed in cells of the caeca, lyrate organ, ganglia, epithelial cells, muscles, and ovaries and within the eggs (Becnel et al. 2002). Infected female mites have a shorter life span and lower fecundity than do uninfected females. Infected mites have male-biased sex ratios (Olsen & Hoy 2002).

Pathogen prevalence in heat-treated colonies (7 days at 33°C) is reduced from 85 to 2%, but the pathogen is not eliminated. Uninfected colonies may be established from progeny mites that are reared from heat-treated females. The majority (about 80%) of adult and progeny mites survive the heat treatment process (Olsen & Hoy 2002), indicating that this method is an effective means of reducing *O. occidentalis* infections in *M. occidentalis.*

25.11.6 *Neoseiulus* (Formerly *Amblyseius*) *cucumeris* (Acari: Phytoseiidae)

The predatory mites *Neoseiulus cucumeris* and *Neoseiulus barkeri* are used for control of western flower thrips, *Frankliniella occidentalis*, and onion thrips, *Thrips tabaci*, on horticultural crops.

Microsporidioses of *N. cucumeris* and *N. barkeri* reduce the quality and number of these predatory mites in mass-rearings (Beerling & van der Geest 1991). Three species of microsporidia infect these predators. The first, thought to belong to the family Pleistophoridae, infects predatory mites (*N. cucumeris* and *N. barkeri*) as well as the forage mites *Acarus siro* and *Tyrophagus putrescentiae*, used as food in production systems. This pathogen has oblong spores that measure $1.8 \times 0.9\,\mu m$. The second microsporidium is found only in prey mites and has spores that measure $1.4 \times 0.8\,\mu m$. The third microsporidium is also observed only in prey mites but only on occasion. Spores measure $2.6 \times 1.3\,\mu m$, and it is speculated that this pathogen is *Nosema steinhausi* (see Beerling et al. 1993), previously described by Lipa (1997) from field-collected *Tyrophagus noxius* from Czechoslovakia and in laboratory colonies of *A. siro*. When infected with *N. steinhausi*, all developmental stages of *A. siro* are filled with spores ($2.16–2.88 \times 1.2–1.44\,\mu m$, fixed and stained).

Diseased predatory mites are swollen, whitish in appearance, and sluggish, and infected prey mites exhibit similar signs of infection although less pronounced. Beerling and van der Geest (1991) speculate the means of pathogen transmission for the pathogen from family Pleistophoridae. Transmission possibilities include vertical transmission and four potential routes of horizontal transmission: (1) transmission as a result of coming in contact with spores that are excreted in feces and/or released from the host upon death; (2) through cannibalism or predation of infected cohorts or prey; (3) through direct contact with infected conspecifics or prey; and (4) through mating. Two cell lines producing monoclonal antibodies have been developed for use in an ELISA to screen mass-reared mites for microsporidia (Beerling et al. 1993).

The microsporidium *Intexta acarivora* was described from the grain mite, *Tyrophagus putrescentiae*, used as food for mass-rearings of *N. cucumeris*. The pathogen is a parasite of the gut epithelia and has round spores with an anisofilar polar filament. Microspores measure 1.3–1.7 μm in diameter, with a polar filament arranged in 2–3 coils, whereas macrospores measure 1.5–2.3 μm in diameter with a polar filament of up to nine coils. The pathology of this pathogen is unknown (Larsson et al. 1997).

25.11.7 *Phytoseiulus persimilis* (Acari: Phytoseiidae)

The predatory mite *Phytoseiulus persimilis* is available for spider mite control on agricultural crops. All developmental stages of *P. persimilis* obtained from a commercial source in Europe were infected with the microsporidium, *Microsporidium phytoseiuli*. Infected mites show no signs associated with infection. Developmental stages of the pathogen are observed within lyrate organ (ovarian tissue), in cells of the cecal wall and in the muscle tissue beneath it. Spores are observed in the lyrate organ and ganglia, within the cecal wall and muscle tissue, in cells underlying the cuticle, and in developing eggs within gravid females (Bjørnson et al. 1996).

Broad- to elongate-ovoid, uninucleate spores measure $5.37 \pm 0.46 \times 2.22 \pm 0.17\,\mu m$ (fixed and stained) and $5.88 \pm 0.34 \times 2.22 \pm 0.19\,\mu m$ (fresh) and have an isofilar polar filament that is coiled 12–15 times within the posterior two-thirds of the spore. The pathogen does not infect two-spotted spider mites, *Tetranychus urticae* (Bjørnson et al. 1996).

A second microsporidium, which is undescribed, was found in *P. persimilis* from a commercial insectary in North America. Uninucleate spores are elongate ovoid, measure $2.88 \times 1.21\,\mu m$, and have a polar filament that coils 7–10 times in the posterior half of the spore. Vegetative stages develop within the cytoplasm of lyrate organ cells, and spores infect numerous tissues and are within developing eggs (Bjørnson & Keddie 2000).

Another undescribed microsporidium was observed in *P. persimilis* from a commercial insectary in Israel. Uninucleate spores are ovoid, measure $2.65 \times 1.21\,\mu m$, and have a polar filament that coils three to four times in the posterior half of the spore. The polar filament and other internal spore characteristics are often concealed by densely packed ribosomes. Vegetative stages of this pathogen are observed within the cytoplasm of cecal wall cells and within the nuclei of lyrate organ cells. Spores occupy cells of numerous tissues and are occasionally seen within the nuclei of lyrate organ cells. The pathogen also infects the ovary and developing eggs (Bjørnson & Keddie 2000).

M. phytoseiuli reduces the fecundity, prey consumption, and longevity of *P. persimilis* females (Bjørnson & Keddie 1999). Vertical transmission of this pathogen is 100%, but horizontal transmission is low and occurs only when immature *P. persimilis* are permitted to develop in contact with other infected immatures or adults. Pathogen prevalence can reach 100% in laboratory colonies (Bjørnson & Keddie 2001). Heat and chemical treatments are unsuccessful for reducing pathogen prevalence in diseased individuals (Bjørnson 1998).

In the case of *M. phytoseiuli*, low disease prevalence accompanied by a lack of obvious disease signs or symptoms increases the probability that these pathogens will escape notice in *P. persimilis* mass-rearings unless individuals are routinely examined for pathogens (Bjørnson & Keddie 2001). In colonies where pathogen prevalence is low, uninfected mites may be isolated from infected ones through the careful examination of individuals for microsporidia. The progeny of uninfected mites may then be used to establish uninfected colonies. When adding field-collected *P. persimilis* to existing colonies, individuals must be screened prior to their inclusion to ensure that existing colonies remain microsporidia-free (Bjørnson & Keddie 2000).

25.11.8 *Sasajiscymnus tsugae* (Coleoptera: Coccinellidae) and *Laricobius nigrinus* (Coleoptera: Derodontidae)

The predatory beetle, *Sasajiscymnus tsugae*, is a natural enemy of the hemlock woody adelgid, *Adelges tsugae*, a pest of hemlock and spruce trees. Originating from Japan, *S. tsugae* was introduced for control of *A. tsugae*, which had been inadvertently introduced into North America from Asia in the 1920s (Reardon & Onken 2004).

Solter et al. (2011) report high mortality in a laboratory-reared colony of *S. tsugae* caused by microsporidiosis. Prevalence of infection in *S. tsugae* increased from 12 to 50% over the course of 1 year. Host specificity trials indicate that under laboratory conditions, the pathogen is capable of infecting several species of predatory beetles that are candidate biological control agents for *A. tsugae*.

One species of microsporidia was isolated from field-collected *Laricobius nigrinus* from Washington and Idaho. The pathogen causes systemic infections resulting in high mortality. Although infected beetles may not show any signs of infection, the microsporidium may reduce fecundity and affect larval development (Solter et al. 2011).

Molecular studies suggest that one species of *Tubulinosema* and several species of *Nosema i*nfect *L. nigrinus*, *S. tsugae*, and the predatory coccinellid *Scymnus coniferarum* that are being mass-reared for *A. tsugae* control. Microsporidia are thought to compromise beetle mass-rearings (Solter et al. 2011).

25.12 OTHER BENEFICIAL INVERTEBRATES

25.12.1 *Steinernema* (=*Neoplectana*) *glaseri* (Rhabditida: Steinernematidae)

Poinar Jr. (1988) reports an undescribed microsporidium from all stages of the entomopathogenic nematode *Neoplectana glaseri*, including the eggs and infective juveniles. Very few microsporidia-infected juveniles emerge and those that do are hyaline and smaller than uninfected *N. glaseri* juveniles. The pathogen affects the development of infective, third-stage juveniles: many have an open intestine and developing gonad rather than the collapsed intestine and primordial gonad exhibited by uninfected juveniles. When infected, the intestinal lumen is frequently filled with microsporidian spores, and juveniles do not survive short periods of storage at 22°C.

Developmental stages of the pathogen and mature spores (mean $2.36 \times 1.16\,\mu m$) are observed in the hypodermis, intestine, and reproductive system (uterus, oviduct, eggs, and testis). Infection often results in partial or complete castration in both sexes, reducing the number of progeny or resulting in the death of the infective juveniles and adults.

Nematodes normally feed *per os* within their hosts; therefore, microsporidia that infect nematodes likely originate from the infected hosts, in this case the cerambycid *Migdolus fryanus* from Brazil. The author concludes that care should be taken to ensure that nematodes are microsporidia-free before introducing them into mass cultures (Poinar Jr. 1988).

25.12.2 *Steinernema carpocapsae* (Rhabditida: Steinernematidae)

Two entomopathogenic microsporidia, *Pleistophora schubergi* and *Nosema mesnili*, also infect the nematode *Steinernema carpocapsae*, but the impact of these pathogens on nematode fitness is unknown (see Kaya et al. 1988).

25.12.3 *Xysticus cambridgei* (Araneae: Thomisidae) and Other Arachnids

There are very few reports of microsporidia in arachnids. In 1960, *Microsporidium weiseri* was found infecting the hemolymph and hemocytes of the harvestman, *Opilio parietinus* (Opiliones: Phalangiidae) collected in Czechoslovakia (see Cokendolpher 1993).

The microsporidium *Oligosporidium arachnicolum* develops in the oocytes and ovarian pedicular cells of the spider *Xysticus cambridgei*. Various developmental stages of the pathogen are grouped closely together in the cell cytoplasm. Spores are oblong, measure 3.6×2 µm, and have a polar filament arranged in seven or eight coils at the base of the spore. Microsporidia may be more common in spiders and other predaceous arachnids than reported because the feeding behavior of these generalist predators may subject them to a variety of microsporidia-infected prey (Codreanu-Bălescu et al. 1981).

25.13 CONCLUDING REMARKS

The cryptic nature of microsporidia and their subtle yet profound effect on host fitness justify the routine examination of both field-collected and laboratory-reared beneficial arthropods for use in biological pest control programs. The observation that microsporidia tend to have more noticeable effects when host insects are under stress is likely the reason why these pathogens are often observed in laboratory and commercial mass-rearings.

There are sound reasons to ensure that microsporidia are eliminated from beneficial arthropods before candidates are tested and/or released as part of a biological control program. Biological control candidates infected with microsporidia may be deemed unsuitable if performance is poor. For example, infected individuals may not be able to survive exposure to their new environment, and fecundity, longevity, or other life history data collected from infected individuals may not accurately reflect the true efficacy of the biological control candidate. The misinterpretation of data from preliminary tests may end the evaluation process prematurely (Goodwin 1984).

Once a microsporidia-infected host has been released, any adverse effects of the pathogen may also have an impact on efficacy, and because microsporidia tend to have a narrow host range, the introduction of exotic microsporidia may present some risk to endemic insects if they are also susceptible to the pathogen (Kluge & Caldwell 1992).

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